

ASPECTS OF THE ECOLOGY AND BEHAVIOUR OF *Hylastes ater* (Paykull)
(Coleoptera: Scolytidae) IN SECOND ROTATION *Pinus radiata* FORESTS IN THE
CENTRAL NORTH ISLAND, New Zealand, AND OPTIONS FOR CONTROL.

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ABSTRACT

This study examined aspects of the pest status and ecology of *Hylastes ater* in *Pinus radiata* reforestation sites.

Aspects of the flight activity, larval survival and adult emergence from stumps by *Hylastes ater* and *Hylurgus ligniperda* were investigated. *H. ater* was found to be univoltine with a peak of flight activity in autumn. Competition from *H. ligniperda* has displaced *H. ater* from sites harvested during the spring and summer months. *H. ligniperda* was bivoltine with peaks of flight activity in spring and summer. The establishment of *H. ligniperda* in New Zealand has resulted in changes in the lifecycle of *H. ater*.

Attack by *H. ater* on *P. radiata* seedlings was found to be the dominant cause of seedling mortality in the first year after planting. Although high mortality of seedlings has been recorded from some sites, seedling mortality greater than 10% was found to be uncommon in this study. However, high levels of sub-lethal feeding were common in many plantings. High-risk sites were those harvested in autumn and planted the following winter. Seedlings treated at planting with the insecticide Marshal suSCon[®] demonstrated a significant reduction in mortality due to *H. ater* attack. Marshal suSCon[®] had a repellent effect on *H. ater*. Sub-lethal attack did not effect the growth of seedlings in the first two years after planting.

Six species of sapstain fungi were isolated from sub-lethally attacked seedlings. There was a significant relationship between severity of attack and invasion of the seedling by sapstain fungi. A seventh species of sapstain was isolated from *H. ater* but was not found in any of the experimental seedlings. Because of the potential economic significance of these findings, further research is required to unequivocally demonstrate whether or not *H. ater* vectors sapstain fungi to seedlings during attacks.

Six unrelated *P. radiata* seedling types were assessed for resistance to *H. ater* attack. Seedling types exhibited varying levels of resistance to attack. The frequency of attack was not different between resistant and non-resistant seedlings, indicating that *H. ater* did not make a selection prior to attack. Further research is required to fully understand the

mechanisms of resistance, but opportunities exist for resistant seedlings to be used in management programmes.

H. ater and *H. ligniperda* were attracted to volatiles in the field. Ethanol added to α -pinene, β -pinene and raw turpentine had a synergistic effect by enhancing attraction. Although there were differences in numbers trapped by the different treatments, sex ratios of *H. ater* attracted to volatiles and the control were equal in number. This indicates that aggregation or sex pheromones are not likely to be produced by *H. ater*, and it is attracted to host volatiles.

The information generated during this research was used to suggest strategies to manage the risks associated with *H. ater* in second rotation forests in New Zealand.

1. GENERAL INTRODUCTION

1.1 Introduction to Scolytidae

Until recently scolytids (bark beetles) have been placed as separate families with platypodids (pinhole borers) due to their morphological, ecological and behavioural similarity (Kirkendall *et al* 1997). However, systematists have been unable to agree whether these groups should be treated as separate families (Wood, S.L. 1973, 1982, 1986 Beaver 1989) or subfamilies of the Curculionidae (Crowson 1968, Kuschel 1990, 1995). Wood, S.L. (1973, 1982, 1986) considers that the Scolytidae and Platypodidae have a separate origin outside the Curculionidae and can be considered separate families. Kuschel (1990), May (1993) and Thompson (1992) consider scolytids and platypodids to be sister taxa placed within the Curculionidae. While the position of scolytids remains unclear, I will use the traditional usage of Scolytidae for the purposes of this research.

Scolytids include over 6,000 species worldwide, with some 1,430 species in North and Central America (Raffa *et al* 1993, Kirkendall 1983, Kirkendall *et al* 1997). In terms of their taxonomy, geographic distribution, relationships with other organisms and general biology, scolytids are a very diverse group of insects and play an important role in natural ecosystems (Neumann 1987, Raffa *et al* 1993). Interactions between bark beetles and their hosts are the culmination of approximately 200 million years of adaptation (Christiansen *et al* 1987, Byers 1995). While most species will only colonise dead, dying and stressed trees, some can attack and kill healthy trees (Rudinsky 1962, Raffa *et al* 1993, Byers 1995). These species have earned bark beetles a reputation as devastating pests. Consequently, bark beetles are known to most biologists by the notoriety of these few tree-killing taxa (Kirkendall 1983, Raffa *et al* 1993).

The Scolytidae are one of the few insect families where the adult can penetrate the protective outer bark of woody plants (Wood, D.L. 1982, Kirkendall *et al* 1997). With the exception of a brief period of short flight, scolytids complete their whole life cycle within the host plant (Rudinsky 1962, Kirkendall *et al* 1997). New hosts are found, then colonised during these flight periods (Gara & Vité 1962, Rudinsky 1962). This habit of scolytids living off the subcortical environment is an evolutionary successful strategy. In

successfully overcoming the barriers to occupying these habitats bark beetles have evolved into a relatively host specific group of insects (Raffa & Berryman 1983). There are ancillary benefits from this specialisation. The subcortical environment provides excellent protection from potential environmental extremes and those natural enemies that lack the specific adaptations required to find subcortical insects (Raffa *et al* 1993). While scolytids have evolved a form to take advantage of every type of plant tissue, most feed inside the inner bark of dead woody plants (Christiansen *et al* 1987, Kirkendall *et al* 1997). Feeding in the inner bark (termed phloeophagy) is likely to be the more evolutionary primitive behaviour (Kirkendall 1983, Kirkendall *et al* 1997). Adaptations are distinct with respect to hosts and parts of the host. Some species may have preferences toward young or old trees of the host species, different portions of the trunk, roots, branches or cones (Rudinsky 1962, Kirkendall *et al* 1997).

Scolytids in general, occupy temporary habitats (Raffa & Berryman 1983, Tribe 1991b, Lindelöw, *et al* 1992) and have adapted to rapidly increase numbers when suitable habitats are found (Tribe 1990, 1991b, Wilson & Day 1995, Kirkendall *et al* 1997, Rieske & Raffa 1999). The ability to find suitable breeding substrate is the limiting factor for all bark beetles in the natural environment (De Jong & Sabelis 1988, Tribe 1991b, Lindelöw *et al* 1992). The young of each generation usually reach their new host by migration as adults rather than minor movements (Southwood 1962, Atkins 1966). This process has been considered expensive for a species, as mortality may be high before new hosts are reached (Kirkendall *et al* 1997). However, Southwood (1962) suggests that the migratory strategy has advantages by enabling a species to move with location changes in its habitat. Such behaviour is required for a species to occupy temporary habitats (Atkins 1966). For scolytids the impermanence in their habitat is a consequence of irregularities in space and time (Atkins 1966, Schroeder 1988). The migratory movement of a species is correlated with the degree of impermanence of its habitat and represents an evolutionary strategy for reductions in disease/predator build-up (Southwood 1962).

A frequently observed feature of bark beetle communities is the diversity and number of associated organisms in the subcortical brood galleries. The four dominant groups are insects, mites, nematodes and fungi. The scolytid subcortical system has been described as a supra-organism, due to the co-evolutionary relationships that exist between bark beetles and the complex of associated organisms (Stone & Simpson 1990). Mite species from 60

families have been recorded associated with bark beetles (Stone & Simpson 1990). These mites use the beetles for transport (called phoresy), feed on the beetles themselves, their broods, or some other component of the beetle gallery habitat (Stone & Simpson 1990, Raffa 1991). This may include nematodes, other mites or detritus. The diversity of the mite-bark beetle association is paralleled by the diversity of bark beetles nematode associates. Fungi from 38 genera have been identified as being associated with conifer killing bark beetles in North America alone (Stone & Simpson 1990). These fungi may play an important role in the bark beetle tree killing strategy (Birch 1978, Coulson 1979, Raffa 1991, Klepzig *et al* 1996a,b, Paine *et al* 1997).

Bark beetles are among the most economically important forest insects (Rudinsky & Vité 1956, Rudinsky 1962, Berryman 1972, Wood, D.L. 1982, Raffa *et al* 1993) and are distributed across a wide range of host trees (Paine *et al* 1997). As the amount of production forestry increases, the potential habitat for these pest species will expand, and may result in increases in economic damage. The killing of live trees by species such as those from the genus *Dendroctonus* is of great economic importance in the Northern Hemisphere (Rudinsky 1962, Raffa & Berryman 1983). Other bark beetles (e.g. members from the genera *Hylastes*) may attack young seedlings, which can be economically damaging when establishing new plantations (Clark 1932a,b, Boomsma & Adams 1943, Rudinsky & Zethner-Møller 1967, Crowhurst 1969, Scott & King 1974, Tilles *et al* 1986a,b, Tribe 1991b, Eidmann 1992, Lindelöw, *et al* 1992).

The subcortical life history of bark beetles means that their chances of surviving the passage from one country to another are good. With increased trade between nations the costs associated with minimising new introductions are high (Alma 1975, Faulds 1989). Bark beetles that attack and kill live trees are not alone in causing large economic damage. Those bark beetles that invade dead trees may also inflict large-scale economic damage by invading recently felled logs and those stored on skid sites (Hosking 1977). The galleries made by these beetles greatly reduce the value of wood and increase the risk of introduction into other countries if the wood is to be exported whole (Bain 1974). Bark beetles often vector their disease and fungal associates (Berryman 1972, Christiansen *et al* 1987, Paine *et al* 1997). While these may not kill trees outright, fungal effects such as sapstain greatly reduce the value of logs (Hertert *et al* 1975, Bedard *et al* 1990, Wingfield & Gibbs 1991, Klepzig *et al* 1996a,b).

1.2 Life History

Scolytids may be characterised according to the physiological substrate they typically colonise (Rudinsky 1962, Wood, D.L. 1982, Raffa 1991, Raffa *et al* 1993, Paine *et al* 1997). Those few species that colonise and kill healthy trees are termed ‘aggressive’ or ‘primary’ species. In healthy trees the phloem and cambium are relatively normal, have a high starch and protein content and normal moisture levels (Rudinsky 1962). Primary or aggressive species are the first organisms to invade plant tissue that is otherwise uninfested and capable of mounting a defensive reaction (Rudinsky 1962, Raffa 1991, Paine *et al* 1997). ‘Non-aggressive’ or ‘secondary’ species are those that colonise dead or dying trees, or trees that may have been previously attacked (Rudinsky 1962, Raffa & Berryman 1983, Raffa 1991, Paine *et al* 1997). The hosts that this group attacks may be mechanically normal, however turgidity is decreased and starch and protein levels are sub-normal. Dead trees characteristically show cambium and phloem discoloration, and moisture levels reaching saturation (Rudinsky 1962).

The terms ‘primary’ and ‘secondary’ refer to the stage in the colonisation sequence in which a species may arrive. In general, this concurs with the aggressive and non-aggressive terminology (Raffa *et al* 1993). Most scolytids colonise dead trees or tree parts. These species are termed saprophytic (Rudinsky 1962, Raffa *et al* 1993). The host plant material of saprophytic bark beetles is generally aged. The cambium and phloem may be discoloured, with evidence of fermentation. Moisture levels are increased and starch and protein levels are low (Rudinsky 1962).

Raffa *et al* (1993) suggest, that this pattern may be complicated by three factors. While each species is characterised by the upper limit of host vigour they may overcome, all species will colonise trees of inferior condition (Eidmann 1992, Mendel *et al* 1992, Paine *et al* 1997). Some species may alter their colonisation behaviour through time. For example, they may be limited to stressed or weakened trees during periods of low population density, but may colonise healthy trees during high-density periods (Raffa & Berryman 1983, Christiansen *et al* 1987, Eidmann 1992, Mendel *et al* 1992). Rudinsky (1962) and Coulson (1979) suggest that systems of grouping bark beetles, such as described here, have not proven to be useful. This is because systems tend to be subjective in defining the nature of host condition, and many species have the ability to exploit a

variety of host conditions. Yet, most authors use a characterisation system similar to that described above (e.g. Wood, D.L. 1982, Raffa *et al* 1993).

The ability to kill healthy trees appears to be a specialised ecological strategy (Raffa *et al* 1993). All scolytids require non-resistant host material for brood production and tree killing is disadvantageous to some individuals. The most obvious risk to individual pioneers is the chance of being killed by host defences (Birch 1978, Borden 1982, Raffa & Berryman 1983). In order to overwhelm host defences large numbers of colonisers are often required. This may result in severe intraspecific competition (Alcock 1982, Borden 1982, 1989, De Jong & Sabelis 1988). While attracting followers is an obvious advantage to pioneers, there must also be some advantages to the followers (Alcock 1982).

Selection for primary attraction and efficient searching mechanisms are critical for an individual's fitness (Alcock 1982, De Jong & Sabelis 1988, Tunset *et al* 1993). While the theory of 'group selection' (Alcock 1982) has been favoured in the past, 'individual selection' is now commonly accepted to govern bark beetle colonisation (Maynard Smith 1989). As an individual pioneer is greatly susceptible to death by host resistance mechanisms, it may not be favourable to be a 'pioneer'. However, a large proportion of a population is reliant on these pioneers to find suitable hosts. If the proportion of pioneers declines below a certain level followers will suffer, as fewer suitable host trees will be found (Tunset *et al* 1993). Therefore, an increase in pioneers will result from the genotype for primary attraction increasing their fitness (Tunset *et al* 1993). Populations therefore operate an Evolutionary Stable Strategy (Maynard Smith 1989). While this strategy has been suggested for bark beetles, further evaluation and modelling is required (Tunset *et al* 1993).

Some pines grow in pure, even-aged, early successional stands and may be more vulnerable to area wide tree killing than other hosts (Raffa *et al* 1993). An advantage of primary or aggressive species is that they can greatly increase the number of available hosts as a function of their own densities (Birch 1978, Borden 1989, Raffa *et al* 1993) resulting in localised areas of host mortality (Birch 1978, De Jong & Sabelis 1988). However, when conditions are unfavourable for large populations, these species may be relatively unsuccessful at competing with less aggressive species for host material with reduced or no defences (Raffa *et al* 1993). This dependence on high numbers to overcome

healthy hosts creates periods of 'feast or famine' and is a consequence of population dynamics and environmental conditions (Birch 1978, Christiansen *et al* 1987, De Jong & Sabelis 1988, Raffa *et al* 1993). These population fluctuations for many scolytids are a function of the availability of suitable food (Rudinsky 1962, Berryman 1972). The ability of primary or aggressive species to overcome healthy hosts may represent an evolutionary shift away from competition, as well as the invasion of a new habitat (Raffa *et al* 1993). While less aggressive species must contend with avoiding resistant hosts, which are often of poorer nutritional quality and greater interspecific competition, they are likely to be able to find some amount of host material at all times (Raffa *et al* 1993). It is possible that individuals from a population may employ different colonisation strategies depending on population levels (Berryman & Ashraf 1970, Raffa *et al* 1993). Some individuals may show greater discrimination toward host selection during periods of low or endemic population levels and are less selective during population outbreaks (Raffa & Berryman 1983).

1.3 The genus *Hylastes* Erichson

The genus *Hylastes* Erichson 1836 is a member of the tribe Hylastini (Wood, S.L. 1982) and occurs widely in the Northern Hemisphere (Lindelöw 1992). The genus is closely related to *Hylurgops* and some species may be taxonomically separated with difficulty (Wood, S.L. 1982). *Hylastes* species are 2.1-6.0mm long, 2.6-3.2 times long as wide, black (may be reddish-brown), breed in the phloem tissues in the stumps, roots and occasionally logs of coniferous trees. For a full description of the genus *Hylastes* refer to Wood, S.L. (1982).

The genus comprises of about 30 species from coniferous forests in the Holarctic region (Wood, D.L. 1982). Twenty-one species may be found in North America (Lindelöw 1992). Five species are found in middle Europe with the same species being found in Britain (Swan 1943). Scott and King (1974) outline six species from Britain, *H. ater* (Payk.), *H. cunicularius* Er., *H. brunneus* Er., *H. opacus* Er., *H. angustatus* (Herbst.) and *H. attenuatus* Er. and suggests the first three are the most important as forest pests.

H. ater and *H. brunneus*, are pests of pines and *H. cunicularius* is found in spruces (Scott & King 1974). These three are the largest species and are morphologically similar. They differ in distribution. *H. ater* in the south is replaced by *H. brunneus* in the north (Scott & King 1974). Beaver (1970) claims *H. ater* and *H. brunneus* are distinct species despite suggestions by others (e.g. Schedl 1968). Due to this taxonomic confusion, changes in species distribution may be expected in the future (Lindelöw 1992).

In Scandinavia, *H. brunneus*, *H. cunicularius* and *H. opacus* are widely distributed (Lindelöw 1992). *H. ater*, *H. attenuatus*, *H. angustatus* are rare and have been reported mainly in the south of Sweden (Lekander *et al* 1977). South of Sweden, *H. ater* replaces *H. brunneus* which is rare in Germany (Reitter 1913) and Poland (Grocholski *et al* 1977). With the exception of a couple of species (i.e. *H. batnensis* Brisout de Barneville), *Hylastes* in Europe are associated with pines (Lindelöw 1992).

Hylastes species have also been unintentionally introduced into other countries. These include *H. angustatus*, *H. linearis* Er. and *H. opacus* into South Africa (Wingfield & Knox-Davis 1980, Wingfield & Swart 1994), *H. ater* into New Zealand (Clark 1932b, Faulds 1989), Australia (Boomsma & Adams 1943, Neumann 1987) and Chile (Ciesla 1988), and *H. opacus* into North America (Bridges 1995).

Hylastes species are best classified as secondary or non-aggressive bark beetles and breed in the roots and stumps of windthrown trees as well as trees killed by other bark beetles (Lownsbery 1988, Lindelöw 1992). Host species include the genera *Pinus*, *Picea*, *Pseudotsuga* and *Abies*. Each species is usually restricted to a specific host genus, but may colonise hosts from other genera (Lindelöw 1992).

Hylastes orientate using host volatiles to locate suitable breeding substrate (Rudinsky & Zethner-Møller 1967, Witcosky *et al* 1987, Eidmann *et al* 1991, Lindelöw 1992). Once adult beetles have located the appropriate breeding material, they enter the roots close to the tree base or burrow down through the soil to enter buried roots up to depths of 100 cm (Forestry Commission 1946, Eidmann *et al* 1991, Lindelöw 1992). Little is known about how *Hylastes* utilise olfactory and visual stimuli and there appears to be no evidence for aggregation pheromones (Eidmann *et al* 1991, Lindelöw 1992). It is not known whether they mass attack or not (Lownsbery 1988). Pine beetles compete with pine weevils for

brood material, but can breed in smaller material (Forestry Commission 1946, Scott & King 1974).

Spring is the main season for breeding. However, oviposition may be delayed in cooler climates. The rate at which a brood may develop is dependent on weather conditions and soil temperature (Forestry Commission 1946, Scott & King 1974, Lindelöw 1992). Eggs laid in spring may take three months to develop through to adults, while those laid during summer may complete their lifecycle in two months (Scott & King 1974). Eggs laid late in the season may overwinter as larvae (Forestry Commission 1946, Scott & King 1974, Lindelöw 1992). The larvae do not usually cause economic damage (Forestry Commission 1946).

Adults may not leave the bark immediately when reaching maturity, if it is still fresh and has not been heavily infested by other broods (Forestry Commission 1946, Scott & King 1974). Parent adults may continue feeding to recuperate and may produce a second brood in the same material (Forestry Commission 1946, Scott & King 1974). If the bark is not suitable food material for a new adult, it must emerge and locate a suitable food supply. Most often this is a young seedling (Munro 1917, Scott & King 1974, Neumann 1987, Lindelöw 1992). While *Hylastes* species are root breeders, they usually concentrate their feeding on the bark and cambium layers of live seedling material (Munro 1917, Eidmann *et al* 1991) and can cause much damage in newly established plantations (Clark 1932a,b, Boomsma & Adams 1943, Swan 1943, Neumann 1987). Young conifers are attacked just below ground level and the beetle usually works its way down into the roots. Seedlings frequently have their taproots girdled and die as a consequence (Forestry Commission 1946). Signs of attack are most common where plantations have been established near or on land where a coniferous crop has been recently felled (Clark 1932b, Swan 1943, Forestry Commission 1946, Neumann 1987). This feeding is widely regarded as being a prerequisite for sexual maturity and is termed 'maturation feeding' (Ciesla 1988, Lindelöw 1992). In addition to killing seedlings, adults may transmit fungal pathogens (Wingfield & Knox-Davis 1980, Lownsbery 1988, Eidmann *et al* 1991, Wingfield & Gibbs 1991, Lindelöw 1992).

1.4 *Hylastes ater* (Paykull)

Hylastes ater is the most common and abundant *Hylastes* species in Britain (Munro 1917, Forestry Commission 1946). *H. ater* is common in every part of Britain where coniferous crops are grown (Forestry Commission 1946). The largest of the *Hylastes* species, *H. ater* is also renowned for causing damage in Britain and Europe (Munro 1917, Forestry Commission 1946) and ranks amongst the most destructive pests (Scott & King 1974).

Distribution

H. ater is a European species and is known from all European countries, from Spain to Russia (Clark 1932b, Swan 1943). It is not recorded in North America (Clark 1932b, Swan 1943). *H. ater* has been unintentionally introduced into, and is established in New Zealand (Clark 1932b, Swan 1943, Crowhurst 1969, May 1993), Australia (Boomsma & Adams 1943, Swan 1943, Neumann 1987) and Chile (Ciesla 1988).

Description and habit of the insect

The adult beetles are 4-5 mm long, 1.4 mm wide and are shining black. They are cylindrical in shape and their sides are approximately parallel (Milligan 1978). The antennae and terminal segments of the legs are a uniform reddish-brown (Milligan 1978). In newly developed (teneral) adults the elytra may retain this reddish-brown colour after other body parts have darkened. The elytra are twice as long as they are wide (Forestry Commission 1946). When viewed from above, a small part of the head is visible from under the pronotum (Milligan 1978). The head projects downwards and is prolonged into a short snout (Milligan 1978). The prothorax is finely punctate with an impunctate median conspicuous ridge (Clark 1932b). The elytra are coarsely punctate (Clark 1932b). The antennae have a seven-jointed funicular and a conspicuous club (Clark 1932b). The males may be distinguished from females by the formation of a less convex ultimate sternite with a conspicuous finely pubescent depression (Clark 1932b). The legs of the female are less hairy than the male, where the lower edge of the tibia has a strong growth of yellow hairs (Forestry Commission 1946).

Adults are strong fliers (Swan 1943), especially in sunlight (Clark 1932b). Adults enter bark at cut ends (in the case of stumps) or burrow through undamaged bark, usually where the bark is in contact with the soil (Munro 1917, Swan 1943, Crowhurst 1969). Their

presence is detected by observing reddish frass when breeding material (i.e. logs) is rolled over (Swan 1943).

The egg gallery or mother gallery is excavated by members of both the sexes, with the female proceeding first, and the male clearing the frass aided by the extra hairs on its body (Clark 1932b, Forestry Commission 1946). The gallery systems do not characteristically contain resinous exudations (Forestry Commission 1946). The mother gallery is vertical or parallel to the axis of the root and consists of a straight tunnel, beginning with a crutch shaped chamber (brood chamber) and is 80-130 mm in length (Munro 1917, Forestry Commission 1946). The tunnel is narrow, with one arm of the crutch longer than the other (Munro 1917). *H. ater* is monogamous and a single mating takes place (Clark 1932b, Crowhurst 1969). Copulation takes place in the crutch chamber and may occur at any time of the year, although is not likely to occur between feeding adults (Crowhurst 1969).

Eggs are laid in evenly spaced niches along both sides of the egg gallery and covered with frass (Swan 1943). Approximately 100 eggs are laid per gallery (Crowhurst 1969) although more are frequently found (Clark 1932b). A notch is excavated at irregular intervals to allow adults to turn (Forestry Commission 1946). The time of initial invasion to oviposition is approximately two to four weeks (Crowhurst 1969). Eggs are ovoid, pearly white up to 0.9 mm long and 0.54 mm in diameter (Munro 1917, Clark 1932b). The eggs show no sculpturing and do not have a micropyle (Munro 1917).

The larva is consistent with Ipid larvae, being legless, white and having a well defined yellowish head with darker jaws (Clark 1932b). It is made of 14 segments including the head (first segment) (Munro 1917). The next three segments make up the thorax and the remaining ten the abdomen. All body segments, except the last two, are similar in size (Munro 1917). *H. ater* larvae can be distinguished from the larvae of all other *Hylastes* species by the presence and position of a tubercle on the epistoma, the extent of submental spines, the size of the setae lateral to the antennae, the width of the labrum and by the length to breadth ratio of the labial palpi (Beaver 1970). For a full description of the larvae of *H. ater* refer to May (1993).

On hatching, larvae feed on the cambium and phloem (Munro 1917, Crowhurst 1969). They begin by boring slender tunnels at right angles to the mother gallery (Swan 1943,

Crowhurst 1969). These tunnels increase in diameter as the larvae grow and begin to radiate in a random manner, interweaving with burrows from other larvae (Munro 1917, Swan 1943, Crowhurst 1969). When the larvae are ready to pupate they cease feeding and construct a pupal cell at the end of the larval galleries and plug the entrance of the cell with frass (Swan 1943, Crowhurst 1969). In the case of the bark being thin, the pupal cell may be burrowed out of the sapwood (Crowhurst 1969).

The pupae are soft and yellow-white, free and show all the parts present in the adult (Munro 1917, Clark 1932b). The head, pronotum and abdomen have conspicuous spines (Clark 1932b). The pupa darkens as pupation advances (Clark 1932b).

H. brunneus is reported to be a variety of *H. ater* (Schedl 1968). However, Beaver (1970) and others (e.g. Duffy 1953, Hansen 1955, Lekander 1965) consider them to be distinct species. For a complete taxonomic description of the adults, pupae, larvae and eggs of *H. ater* refer to Munro (1917), Clark (1932b), Crowhurst (1969), Beaver (1970) and May (1993).

Life history and development

In Britain Munro (1917) reports *H. ater* has two broods per year. In New Zealand there appears to be three broods per year, which overlap to a certain extent, with the later developing pupae of one brood coinciding with early pupae of the next (Clark 1932b, Crowhurst 1969). The development of feeding larvae is slower in winter than in the summer months and larvae may spend several months resting after the completion of feeding prior to pupation (Clark 1932b, Crowhurst 1969). Both adults and larvae may overwinter and in some cases the larvae may be fully fed (Clark 1932b). Adults, eggs, larvae and pupae are present all year round in New Zealand (Clark 1932b, Crowhurst 1969). However, in Britain eggs and pupae are present only at certain times of the year (Munro 1917). The difference in the life cycle between *H. ater* in the Northern and Southern Hemispheres has not been properly explained.

In Britain adult beetles take six to seven weeks to cut galleries and lay eggs (Munro 1917). In New Zealand eggs are usually found two to four weeks after the adult beetle invades host material. However oviposition has been recorded one week following invasion, and may take up to ten weeks (Clark 1932b). Eggs take two to three weeks to hatch, and larvae

then feed for six to seven weeks (Munro 1917, Clark 1932b). In Britain larvae spend two to three weeks resting after feeding before pupating (Munro 1917). In New Zealand this resting stage also takes from two to three weeks in summer, but up to several months in winter (Clark 1932b, Crowhurst 1969). The pupal stage is short and lasts one to two weeks (Munro 1917, Crowhurst 1969). The development from egg to adult is reported to take two to three months in Britain (Munro 1917). In New Zealand development varies from two months to ten months depending on temperature (Clark 1932b, Crowhurst 1969, May 1993).

Recorded host species

In Britain and Europe, *H. ater* has been recorded from *Pinus sylvestris* L., *P. nigra* Arnold (=laricio), *P. strobus* L., *Picea sitchensis* (Bong.) Carr., *Larix decidua* Miller (=europaea), *Pseudotsuga menziesii* (Mirb.) Franco (=taxifolia) and *Abies nordmanniana* (Steven) Spach (=pectinata) (Clark 1932b), but is more commonly found attacking members of *Pinus* (Forestry Commission 1946, Scott & King 1974).

Damage

While *H. ater* breeds in stumps and roots or dead and dying trees, economic damage is caused when adults maturation feed on both naturally regenerating and planted seedlings (Clark 1932b, Boomsma & Adams 1943, Forestry Commission 1946, Scott & King 1974, Ciesla 1988). In natural conditions *H. ater* has not been recorded as a pest species. However, in second rotation forests, populations do reach epidemic/economic levels (Clark 1932b, Crowhurst 1969). Trees up to six years old in Britain and ten years old in Germany are reported to have been destroyed (Clark 1932b). Some of the damage in European countries may be confused with, and attributed to other bark beetles (Forestry Commission 1946, Scott & King 1974, Lindelöw 1992). The countries where only *H. ater* is found provide good evidence of its potential as a pest, as *H. ater* attacks can be confused with feeding by other bark beetle species. In Australia 90% of seedlings in one 40 hectare compartment were dead or dying following *H. ater* feeding, while 6,000 seedlings in another compartment were reported to be attacked by *H. ater* (Boomsma & Adams 1943). Neumann (1987) reported that all one-year old seedlings in a 3.2 hectare area in Victoria, Australia were killed by *H. ater*. In Chile seedling mortality up to 70% in naturally regenerating areas and 65% in planted areas has been recorded (Ciesla 1988).

Associated insects and other organisms

In Britain, Munro (1917) catalogues the insects associated with *H. ater* into three classes, pests, predators and parasites, and those insects that are neither harmful nor beneficial. The last group was unidentified dipteran larvae (Munro 1917). Many other invertebrates, for example, mites and nematodes are associated with *H. ater* (Dowding 1973, 1984). However the occurrence of these is incidental in most cases and has little influence on *H. ater*.

Other pest species associated with *H. ater* in Britain include *Hylobius abietis* L., *Pissodes pini* L., *Tomicus* (*Myelophilus*) *piniperda* (L.), *Hylastes brunneus*, *H. angustatus*, *H. attenuatus* and *Hylurgops* (*Hylastes*) *palliatum* (Gyll.) (Munro 1917, Forestry Commission 1946, Dowding 1973, Scott & King 1974). *P. pini*, *T. piniperda* and *H. palliatum* are rarely found in the roots and are more consistently associated with the upper portions of the stump (Munro 1917).

In Australia *H. ater* is associated with *H. ligniperda* (Swan 1943, Lawson & Morgan 1993) and occasionally *Ips grandicollis* (Eichhoff), a North American bark beetle (Munro 1926). In Chile, *H. ater* is known to occur in *Pinus radiata* plantations with the bark beetles *Hylurgus ligniperda* and *Orthotomicus erosus* Wollaston (both native of Europe) (Ciesla 1988). In South Africa, *H. ater* is associated with the bark beetles *Hylastes opacus*, *Hylurgops palliatum* and *Tomicus piniperda* L. (Wingfield & Gibbs 1991).

Predatory and parasitic insects associated with *H. ater* in Britain are more numerous and belong to coleopteran and hymenopteran groups (Munro 1917). Commonly found species from six coleopteran genera include, *Thanasimus* (*Clerus*) *formicarius* (L.), *Rhizophagus dispar* (Payk.), *R. ferrugineus* (Payk.), *R. depressus* (F.) *R. bipustulatus* (F.) and *Tachyporus chrysomelinus* (L.) (Munro 1917, Faulds 1989), and *Glischochilus quadripunctata* L. (Dowding 1973).

The parasitoids *Roptrocercus xylophagorum* (Ratzeburg) (Torymidae) and *Dendrosoter sulcatus* (Muesbeck) (Braconidae) and the predators *Thanasimus dubius* (F.) (Cleridae) and *Temnochila virescens* (F.) (Ostomidae) have been introduced into Australia from North America for the purpose of controlling *Ips grandicollis*. However they also predate *H. ater* and *H. ligniperda* (Neumann 1987, Lawson & Morgan 1993).

The fungi *Leptographium lundbergii* Largerburg and Melin, *Ophiostoma* (= *Ceratocystis*) *piceae* (Münch) H. & P. Sydow, *O.* (= *Ceratocystis*) *penicillatum* (Grossm.) and *Graphium aureum* Corda are associated with *H. ater* in Britain (Dowding 1973, Wingfield & Gibbs 1991). *Thanasimus formicarius* may transfer spores of *Ophiostoma* (= *Ceratocystis*) *coerulescens* (Münch) Nannf. to *H. ater* (Dowding 1973). Five *Leptographium* and two *Graphium* species are associated with bark beetles in South Africa. Of these, *Leptographium serpens* (Goid.) Siem. and an unidentified *Graphium* species have been found on *H. ater* (Wingfield & Gibbs 1991). In Chile localised secondary attacks by bark beetles have been found in the root zone of trees infected with the root pathogen *Verticicladiella* (= *Leptographium*) species, although vector relationships have yet to be established (Ciesla 1988).

Control

In Britain and Europe natural predators and parasitoids fail to control epidemic populations of *H. ater*, and other bark beetles and bark weevils (Clark 1932b, Forestry Commission 1946, Scott & King 1974, Salisbury & Leather 1998). The earliest attempts at protecting seedlings from *H. ater* were to remove or burn any slash, and to trap beetles by laying out billets and destroying them (Munro 1917, 1926, Clark 1932b, Forestry Commission 1946). While these techniques may be theoretically effective, in practice burning slash and stumps is unlikely to destroy enough habitats for effective control. The trapping of beetles requires too many resources over large plantation areas (Boomsma & Adams 1943, Swan 1943) to be economically effective. The failure of these simple techniques is evident today by the continued presence of the problems associated with bark beetles and weevils.

Delaying plantings for two to four years has been suggested as an effective silvicultural technique to control *H. ater* (Swan 1943, Scott & King 1974). This allows for the exhaustion of potential habitat. However, the loss of yield and the deterioration of the site usually make this technique unacceptable, except in areas where enormous beetle populations have built up (Scott & King 1974). Other silvicultural operations such as ripping, ploughing and stump removal may reduce the quality of potential habitat (Scott & King 1974).

More recently, chemicals have been used to effectively control *H. ater* and other bark beetles (Scott & King 1973, 1974, Du Toit 1975, Lemperière & Julien 1989, Mrlina *et al*

1994). However, the cost of insecticides and any additional application costs restrict their use operationally. Salom *et al* (1994) and others (e.g. Zúñiga & Stary 1995, Salom *et al* 1996, Klepzig & Schlyter 1999) have suggested the use of repellents and antifeedants in the soil, with little evidence of operational success.

The lack of effective natural predators in epidemic situations in Britain and Europe means that there is little opportunity for biological control in these countries (Scott & King 1974), although there may be potential for control using predators and parasites in integrated pest management programmes (Leather *et al* 1999). Bark beetle predators from North America have been introduced into Australia for the control of *Ips grandicollis* and it is thought that these may reduce *H. ater* populations (Neumann 1987, Lawson & Morgan 1993).

1.5 *Hylastes ater* in New Zealand

H. ater was first discovered in New Zealand in 1929, at Foxton in *Pinus radiata* D. Don (Clark 1930, 1932b). The insect was quickly identified and a survey was carried out to ascertain the extent of the infestation. *H. ater* was considered to have infested too large an area to attempt complete eradication (Clark 1930). A survey later showed that the *H. ater* was already established in a large number of districts at this time (Clark 1932b). By 1932, *H. ater* was recorded in both the North and South Islands of New Zealand, from the central and lower North Island, and from Nelson to Canterbury in the South Island (Clark 1932b). The potential pest status of this insect was recognised at this time, and a study of its behaviour and damage potential was undertaken (Clark 1932b). However, New Zealand was in an economic depression at the time and many experiments were abandoned due to the expense involved (Clark 1932b).

The experiments that were completed form the basis of what is understood about *H. ater* in New Zealand. For example, notes on the life history in the field, breeding experiments to determine the preferred substrate, the host species attacked in New Zealand, distribution, mode of attack, factors influencing outbreaks and techniques for control (Clark 1932b). *H. ater* was only considered to be causing significant damage in Foxton, where it had caused severe losses in a young pine stand (Clark 1932b).

It was recognised that consecutive thinning and a build-up of slash provided breeding material to maintain a reasonable population of *H. ater*. Following harvesting, population levels were able to build rapidly, with emerging beetles attacking young seedlings (Clark 1932b). Weak seedlings appeared to be more susceptible (Clark 1932b). Billet trapping was acknowledged as a means of controlling outbreaks in small stands. However, the potential of this technique in larger areas was not evaluated (Clark 1932b). Clark (1932b) discussed the potential of biological control and recommended that a programme be initiated. This would have allowed populations of the controlling agent to be established throughout New Zealand before coniferous forests were extensively utilised (Clark 1932b).

By 1935, it was expected that *H. ater* would become widespread and billet trapping would be unable to control the pest over large areas (Miller 1935). Biological control was considered most appropriate at this time. Three species *Rhizophagus ferrugineus*, *R. dispar* and *R. bipustulatus* were thought to be most suitable for transportation and a shipment of 3,711 individuals was sent from Britain and released in the main areas of *H. ater* infestation (Miller 1935). A few individuals were kept for breeding (Miller 1935).

From 1935 there is little information documenting *H. ater*, until in 1956 severe mortality of natural regeneration was reported from Kaingaroa State Forest (Zondag 1956). *H. ater* was reported to be a factor contributing to seedling death and was considered to be a serious problem. Beetles were found breeding in stumps up to three years after felling (Zondag 1956). In areas of low populations, beetles were found five years after felling (Zondag 1956). Populations in New Zealand were reported to be large, with larger populations quickly exhausting food supplies (Zondag 1956). Zondag (1956) suggested that biological control should not be further investigated, as controlling agents would ensure that low-level populations persist for longer periods.

During 1956 and 1958 a study was initiated to determine the influence of factors considered detrimental to the establishment of *Pinus radiata* in New Zealand (Zondag 1958). *H. ater* was the main factor contributing to seedling mortality, with frost damage increasing the susceptibility of seedlings to *H. ater* attack (Zondag 1958). Mortality due to *H. ater* was found to differ with respect to aspect (Zondag 1956). Maturation feeding occurred on the roots of larger seedlings (up to 1.2 m tall) as well as small seedlings. However, larger seedlings seldom died following attack (Zondag 1958). Increased

harvesting resulted in *H. ater* populations reaching serious levels (Zondag 1958). Zondag (1958) suggested that populations were so high that he could not stand by earlier advice discouraging biological control, and urged that serious consideration be given to investigating biological control options. The three nitidulid predators (*Rhizophagus ferrugineus*, *R. dispar* and *R. bipustulatus*) released for biological control in the 1930's had not become established (Zondag 1958). Control of *H. ater* at this time centred on insecticide use. Insecticides were either applied to the roots prior to planting, or sprayed onto natural regeneration (Zondag 1958).

Following Zondag's reports there was little mention of *H. ater* in the New Zealand literature. Rawlings (1959) gave a presentation to the Tenth International Congress of Entomology on entomological and other factors influencing *P. radiata* plantations and suggested he did not consider *H. ater* a threat to New Zealand forestry. However, Zondag (1964, 1965) suggested *H. ater* was the most troublesome pest of *P. radiata* regeneration and could cause severe seedling mortality of up to 50% in some areas.

In the late 1960's *H. ater* received further attention, with reports by Dugdale and Zondag (1966) and a substantial study by Crowhurst (1969) on the breeding behaviour of *H. ater* at Eyrewell Forest, indicating the seriousness of the pest. However while a previous supporter of *H. ater* as a serious pest, Zondag (1968) doubted the importance of *H. ater* and suggested that *H. ater* selected weak seedlings to attack. Zondag (1968) stated that logs destined for export needed to be free from *H. ater*.

Crowhurst (1969) was the first to extensively study *H. ater* in New Zealand and confirmed Clark's (1932b) observations on life history, expanding on aspects of larval development. Crowhurst (1969) undertook a detailed survey to search for evidence of the three *Rhizophagus* species released in 1933 and failed to recover any of the species released.

In 1971 a number of studies were initiated in compartment 12g, Karioi State Forest. At this time there seemed to be confusion with regard to the pest status of *H. ater*. Following a visit to compartment 12g in 1971, Milligan (1971a,b) suggested that previous claims of widespread seedling mortality attributable to *H. ater* were inconclusive and the presence of the beetle on dead seedlings did not necessarily infer that they were the cause of death. This was despite a trial at Eyrewell Forest in 1967 showing 1% of insecticide-treated

seedlings were attacked by *H. ater* and survived, while 40% of unprotected seedlings were attacked with 20% mortality (Bain 1971). A survival assessment of compartment 12g carried out in early February 1971 showed 84% survival but by March this figure had dropped to 66% and *H. ater* were observed feeding on 80% of seedlings (Milligan 1971a).

As far back as 1970 it was demonstrated that pre- or post-planting insecticide treatments enhanced seedling survival when *H. ater* populations were present (Milligan 1971a). It was popularly believed that the amount of root growth one year after planting and the amount of resin produced by seedlings in response to *H. ater* attack were good indicators of tree health (Milligan 1971a). The hypothesis that healthy seedlings were resistant to successful *H. ater* attack was developed. Research carried out by Milligan (1971a) refuted this. Milligan (1971a) formed four classifications based on this hypothesis: 1. Root growth since planting (RGSP) and producing resin 2. Root growth since planting (RGSP) no resin 3. No root growth since planting (NRGSP) producing resin 4. No root growth since planting (NRGSP) no resin. He tested this hypothesis in Karioi and Kaingaroa Forests.

If the premise that root growth since planting (RGSP) and resin response to wounding gave immunity to *H. ater* attack were true, it would have been expected that most trees attacked by *H. ater* would have fallen into the category 'NRGSP no resin', and to a lesser extent, into categories 2 and 3 as outlined above. Conversely few trees would be in Category 1. Milligan's (1971a) results showed that this was not borne out by the analysis. He found that 26.6% of seedlings in Karioi Forest and 39.1% of seedlings in Kaingaroa Forest which were attacked by *H. ater* were from Category 1 (RGSP and resin). Although it is reasonable to assume that pests attack the least resistant (stressed) plants first, this does not mean they will not attack more vigorous plants if they are the only plants available. The suggestion that *H. ater* damage will not occur if only Category 1 plants are planted appears to be largely based on anecdotal information.

Reports were still being made throughout the 1970's that *H. ater* did not kill healthy trees and that the seedling mortality attributable to *H. ater* was minimal (Bain 1973, 1980). However, the likely presence of *H. ater* in log exports was a problem (Hosking 1977, Zondag 1979, 1982). To reduce the amount of export log fumigation, a biological control programme was initiated with the intention of reducing bark beetle populations in New Zealand (Zondag 1979).

In 1975, two pteromalid parasitoids (*Rhopalicus tutele* (Walker) and *Dinotiscus eupterus* (Walker)) were introduced from Europe (Scheibelreiter 1976, Zondag 1976b, 1979, Zondag *et al* 1976). While the breeding of *D. eupterus* failed initially, breeding *R. tutele* was more successful (Zondag 1976b, Zondag *et al* 1976). When *R. tutele* was released, there was little hope of it becoming established. Most *H. ater* larvae bred in areas of the stump where the parasitoid was unable to reach them (Zondag 1976b, 1979). Further introductions of these two species were made from Europe and a colony was kept for a short period of time. However, no further releases were made (Zondag *et al* 1976). There was no evidence for the establishment of *R. tutele* and if it had become established it was unlikely to successfully control bark beetle populations (Faulds 1989).

In 1976, a clerid predator, *Thanasimus formicarius* was introduced into New Zealand from Austria (Zondag 1979, Faulds 1989). Between 1977 and 1987, 12 385 individuals were released into the field (Faulds 1989). However, as *T. formicarius* and *H. ater* did not share the same habitat, control was unlikely to be successful (Faulds 1989). Recommendations were made to cease *H. ater* control attempts and its role in seedling mortality was again being questioned. Populations were not likely to be reduced to levels where significant reductions in export fumigation costs could be made (Faulds 1989).

It is apparent from the New Zealand literature that the role of *H. ater* in seedling mortality has been controversial. Seedling mortality (up to 50%) as a result of *H. ater* damage is still being reported (Forest Research Institute unpublished reports). With increasing reports of mortality due to *H. ater* damage in second rotation forests it is appropriate to re-visit this problem and produce management and control regimes that will reduce the costs associated with 'blanking' (filling in) in susceptible areas.

Recorded host species

In New Zealand *H. ater* has been recorded attacking the following species: *Pinus radiata*, *P. muricata* D. Don, *P. pinaster* Ait., *P. ponderosa* Doug., *P. nigra*, *P. patula* Schiede & Deppe, *P. sylvestris*, *P. taeda* L., *P. contorta* Doug., *Sequoia sempervirens* (D. Don) Endl. and *Chamaecyparis lawsoniana* (A. Murray) (Clark 1932b, Miller 1935, Dugdale & Zondag 1966). *H. ater* has also been recorded attacking apples in the North Island (Clark 1937).

Associated insects and other organisms

Two species of Scolytidae are commonly associated with *H. ater* in pine host material. These are *Pachycotes peregrinus* (Chapuis) and *Hylurgus ligniperda*. *P. peregrinus* is a native species which attacks softwoods of economic importance in New Zealand (Bain 1977b). Distributed throughout New Zealand, except in dry areas and superficially resembling *H. ater*, *P. peregrinus* differs from *H. ater* and *H. ligniperda* by boring into the wood of the stump (Bain 1977b). *P. peregrinus* is also frequently found in stumps of *Pseudotsuga menziesii* (Alma & van Boven 1976). *H. ligniperda* is widely distributed in Europe, the Mediterranean area and the Atlantic Islands and has been accidentally introduced into Japan, South Africa, South America, Sri Lanka and Australia (Bain 1977a). It was accidentally introduced into New Zealand in 1974 and is now widespread (Bain 1977a). *H. ligniperda* is restricted to pine in New Zealand (Bain 1977a). *H. ligniperda* superficially resembles *H. ater* and occupies a similar habitat. Both *H. ligniperda* and *P. peregrinus* are of economic importance, by inhabiting logs destined for export, which then require treatment (Bain 1977a, Zondag 1979, 1982, Faulds 1989).

New Zealand has few other bark beetles. These include *Phloeosinus cupressi* Hopkins, and the ambrosia beetles *Xyleborus truncatus* Erichson and *X. saxeseni* (Ratzeburg). *P. cupressi* originates in California and is recorded from *Cupressus* species and *Chamaecyparis lawsoniana* in New Zealand (Zondag 1976a). *X. saxeseni* attacks more than 30 species of softwoods and hardwoods in New Zealand, including *P. radiata*, by boring into the wood (Hosking 1979). It is distributed across Europe, Asia and North America (Hosking 1979). *X. truncatus* is originally from Australia and breeds in *Eucalyptus* species, and a few native species in New Zealand (Hosking 1979).

A number of Coleoptera and Diptera species inhabit *P. radiata* stumps. While some Coleoptera may be predatory on bark beetles, they do not appear to greatly influence populations. The relationship of other species is likely to be more incidental, and of little relevance to this review.

The root disease fungi *Leptographium truncatum* (Wingfield & Marasas) Wingfield and *L. procerum* (Kendrick) Wingfield have been found to be associated with *H. ater* and *H. ligniperda* in New Zealand (MacKenzie & Dick 1984, Wingfield & Gibbs 1991).

Wingfield and Gibbs (1991) suggest *L. procerum* may have been introduced into New Zealand with either *H. ater* or *H. ligniperda*.

Four species of nematode have been associated with *H. ater* in New Zealand (Dale 1967). These four species have been recorded from *H. ater* in Germany, but seven other species associated with *H. ater* in Germany have not been recorded here (Dale 1967). No New Zealand nematode appears to have occupied the niches of the absent German species, but one New Zealand species *Anguilluloides zondagi* n. is consistently recorded from the frass of *H. ater* (Dale 1967).

1.6 The objectives of this research project

Little detailed documentation has been readily available with respect to the impacts of *H. ater* in New Zealand. Forestry practices have changed dramatically over recent years. Perceptions of the pest status of *H. ater* have also changed over time. It is likely that changes in forestry techniques and practices have affected the biology of *H. ater*. The recent introduction of *H. ligniperda* may have had some effect on *H. ater*. Potential interactions between *H. ater* and *H. ligniperda* may have impacted on the pest status of *H. ater*. It is an appropriate time to reassess the threat of *H. ater* to forestry in New Zealand. The primary objective of this research was to determine the impacts of *H. ater* on the New Zealand *P. radiata* forest industry, and recommend management strategies to minimise effects of the pest.

This primary objective has been addressed in two parts. First, aspects of the ecology and behaviour of *H. ater*, and any interactions with *H. ligniperda* were investigated. An understanding of those factors that influence *H. ater* populations in different sites, and during different time periods (or seasons) may provide valuable information to help understand the pest potential of *H. ater*.

The second part of this thesis investigated the impacts of *H. ater* in central North Island forests. Seedling mortality resulting from *H. ater* attacked was quantified, as well as the amount of sub-lethal attack by *H. ater*. The effect of sub-lethal attack on seedling health was examined. Options for controlling *H. ater* were investigated. In areas where control

may be unsuitable, other strategies to manage or reduce the impact of *H. ater* were addressed.

Finally, the information generated from the research undertaken during this thesis was used to develop a preliminary risk management strategy. In doing this the potential techniques for ameliorating the effects of *H. ater* were evaluated. Attempts were made to investigate a strategy to effectively manage the *H. ater* problem, while having minimal impacts on current forest practices and the environment.

2. OBSERVATIONS ON ASPECTS OF THE ECOLOGY AND BEHAVIOUR OF *H. ater* AND *H.* *ligniperda* IN SECOND ROTATION *P. radiata* FORESTS IN THE CENTRAL NORTH ISLAND, NEW ZEALAND

2.1 A preliminary investigation of *H. ater* and *H. ligniperda* larval populations

2.1.1 Introduction

Clark (1932b) and more recently Crowhurst (1969) observed the breeding behaviour of *H. ater* in New Zealand *P. radiata* forests. Significant differences between observations made by them were as follows. Crowhurst (1969) recorded all life stages of *H. ater* at all times of the year. Clark (1932b) stated that eggs were found from November to June, and pupae were present in October and November and from January to April. Other than these differences, observations made by Clark (1932b) and Crowhurst (1969) were consistent. A summary of the lifecycle of *H. ater* was presented in Chapter One. The development of *H. ater* in New Zealand described by Crowhurst (1969) is as follows:

H. ater produces broods throughout the year and all stages of development (egg, larvae, pupae and adult) are present at all times (Crowhurst 1969). The shortest development period from egg to adult was six weeks for eggs oviposited between February and mid-April (Crowhurst 1969). Eggs laid at other times take longer to develop to maturity (up to ten months) (Crowhurst 1969). Longer development times are due to larvae overwintering (termed 'diapausing'). These diapausing larvae are members of slow-developing broods that complete development by January (Crowhurst 1969). Adults from fast-developing broods also mature in January; therefore the offspring produced by these adults will develop during the period best suited for fastest development (Crowhurst 1969). Both fast and slower broods reach maturity at the same time, thus adults from both broods are not segregated (Crowhurst 1969). Eggs oviposited from winter to end of summer take between

three and six months to complete development (Crowhurst 1969). Clark (1932b) and Crowhurst (1969) observed three overlapping generations in the field.

While neither Clark (1932b) nor Crowhurst (1969) identified periods of peak adult activity, indications from Crowhurst (1969) were that most adults reached maturity during January. It is probable that the greatest number of adults were present at this time. Neither Clark (1932b) nor Crowhurst (1969) indicated the relative numbers of individuals present at other times of the year.

Since the observations made by Clark (1932b) and Crowhurst (1969), *Hylurgus ligniperda* has become established in New Zealand. *H. ligniperda* was first discovered in New Zealand in 1974 (Bain 1977a) and has since become widely established. *H. ligniperda* is distributed across Europe, the Mediterranean, and the Atlantic Islands. It has been introduced into Japan, South Africa, South America, Sri Lanka and Australia (Bain 1977a).

H. ligniperda breeds in stumps, logs and roots of pine species. In New Zealand it is mainly found in *P. radiata* (Bain 1977a). The adult beetles superficially resemble *H. ater*, but are larger and are a reddish-brown colour. Golden hairs cover most of the surface of *H. ligniperda*. *H. ligniperda* occupies the same breeding habitat as *H. ater*. The brood gallery is established by the female and is similar to (but longer than) the gallery established by *H. ater*. Eggs are laid over a six-week period in batches along the brood gallery. Periods of oviposition are followed by periods of feeding, followed by more oviposition (Bain 1977a). Development from the initiation of brood galleries to the emergence of adults during the summer months takes around ten to eleven weeks. The rates of development of *H. ligniperda* and *H. ater* are similar (Bain 1977a). Unlike *H. ater*, adult *H. ligniperda* does not attack seedlings following emergence (Bain 1977a).

An important part of assessing the importance of *H. ater* to the *P. radiata* forest industry in New Zealand is to develop a better understanding of its life history. The first part of this study examined interactions with *H. ligniperda*. Preliminary observations of the composition of stump larval populations enabled strategies to investigate the ecology and behaviour of *H. ater* and *H. ligniperda* to be developed.

The objectives of this thesis research were to:

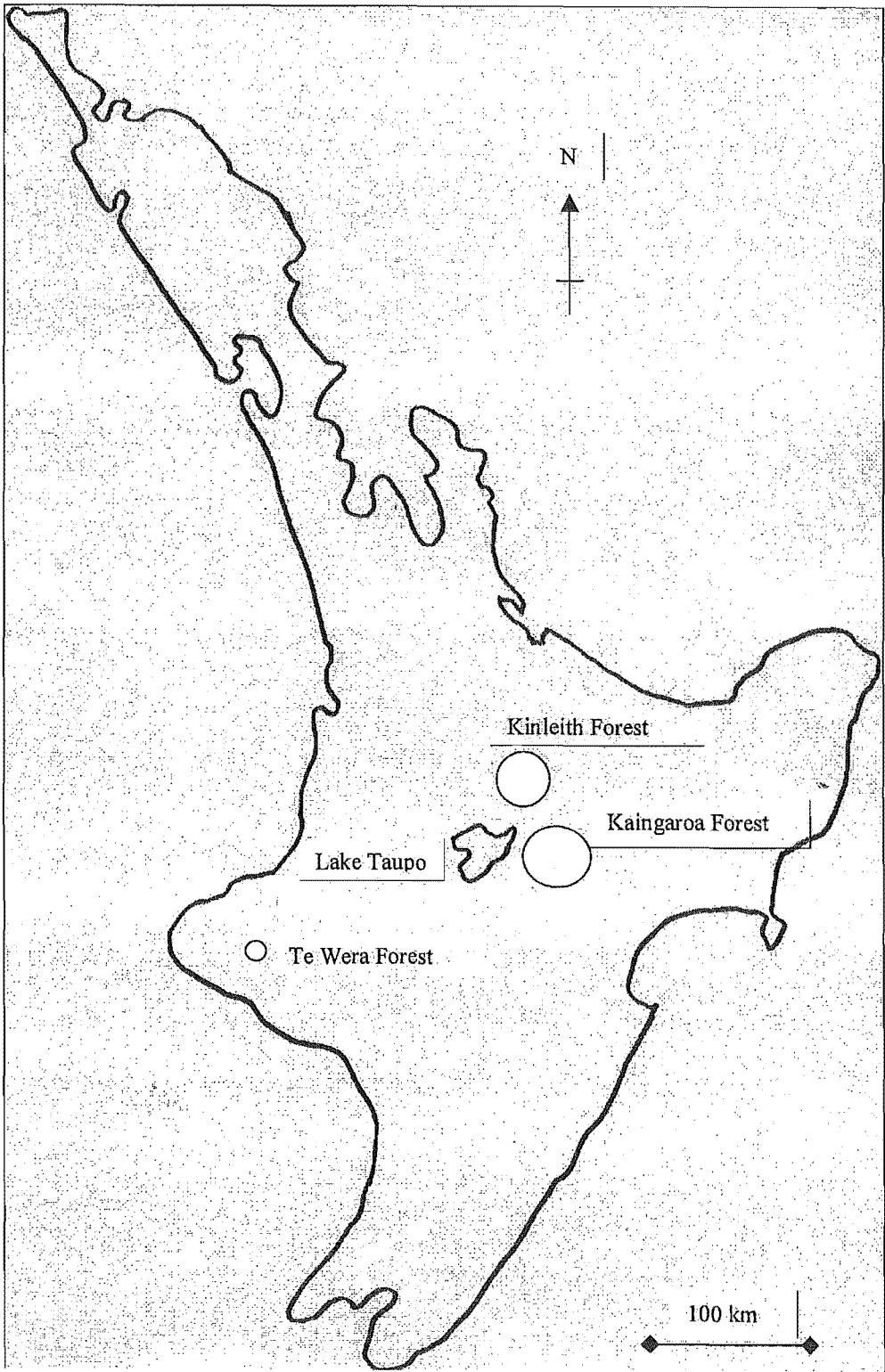
- Determine whether *H. ater* and *H. ligniperda* occupy similar habitats
- Investigate whether the two species co-exist in the same habitat
- Investigate potential differences in population sizes of both species between sites
- Identify factors that may influence relative population sizes
- Evaluate whether the larval populations of each species are influenced by similar factors
- Develop a strategy to further assess the ecology and behaviour of *H. ater*

2.1.2 Methods

During October 1997, twelve sites were selected in two second rotation *P. radiata* plantation forests (Figure 2.1, Appendix 1). Kinleith Forest (Carter Holt Harvey Forests) covers approximately 129,000 hectares and consists almost entirely of *P. radiata* plantation forest. Kaingaroa Forest (Fletcher Challenge Forests) includes 144,000 hectares of *P. radiata* plantation forest.

The soils of Kaingaroa and Kinleith Forests are derived predominantly from volcanic ash from numerous eruptions over the last 20,000 years (New Zealand Soil Bureau 1968, Rijske 1994). The thickness of the volcanic ash, compaction, differences in nutrient levels as well as geographic features such as slope and aspect, contribute to most of the variation in soils (Rijske 1994). Yellow-brown pumice soils are the most extensively and frequently occurring soils in these forests and are formed from Taupo pumice erupted between 500 and 5,000 years ago (New Zealand Soil Bureau 1968, Rijske 1994). The planting of exotic trees on these soils has made the soils physically better for plant growth, due to root penetration (New Zealand Soil Bureau 1968). Other soils of these forests include central recent soils, central yellow-brown loams and steepland soils related to the three soil groups (New Zealand Soil Bureau 1968, Rijske 1994). The factors that may limit tree growth in these forests are a cool climate at higher altitudes and physical barriers to root growth (Rijske 1994).

Figure 2.1 Location of forests in central North Island, New Zealand.



Annual rainfall is approximately 1 600 mm (Quayle 1984). The highest rainfall occurs during May to August, while the driest conditions are generally between November and February (Quayle 1984). Droughts and dry spells are common during the summer months. Thunder and hail may occur at any time of the year but are more likely during the winter months, while snow is rare (Quayle 1984). The prevailing winds are west to southwesterly. Gales are infrequent but do occur, mostly from the northeast and southwest (Quayle 1984).

Temperature variations (both seasonal and diurnal) are relatively small due to New Zealand being a small landmass surrounded by ocean. Mean daily maximum temperature is over 20°C during the summer period. The mean temperature during winter months is approximately eight degrees Celsius. Inland areas are subject to cooler night time temperatures during winter and frosts are common (Quayle 1984). The area experiences approximately 1 950 sunshine hours per annum (Quayle 1984).

Sites were selected if they had been harvested at the end of summer (February to April) 1997. This meant there was a six to nine month period for larval development from time of oviposition. It was expected that stumps in these sites would contain overwintering populations of bark beetle larvae, either *H. ater*, *H. ligniperda*, or both. An assumption was made that the development of larvae would be similar in each site. A few stumps in each site were examined prior to the initiation of this study to ensure larval populations were present.

A transect was randomly located within each site. Every third stump intercepted was selected for sampling. Twenty stumps were sampled in each site, 240 stumps sampled overall.

For each stump selected the following variables were recorded:

Stump height

The height of the stump was measured from the top of the main roots (below the soil surface) to the top of the stump.

Stump diameter

The diameter of the stump was measured across the top of the stump.

Outer bark area

On some occasions heavy harvesting machinery had damaged stumps. This often resulted in areas of bark being knocked off the stump. The amount of bark remaining on the stump (above the main roots) was estimated as follows:

1= 0 - 20 % bark remaining

2= 21 - 40 % bark remaining

3= 41 - 60 % bark remaining

4= 61 - 80 % bark remaining

5= 81- 100 % bark remaining

Inner bark condition

The condition of the inner bark (or cambium layer) is a reasonable indicator of the amount of food resources available for bark beetle broods. The amount of cambium was estimated on a five-point scale. A score of 1 represented little or no cambium, evidence of frass and galleries indicated that the condition of the stump had greatly deteriorated. A value of 5 indicated that the cambium was in excellent condition and no evidence of feeding by bark beetles or other insects was evident.

Slash

Slash is waste left after harvesting operations (i.e. broken branches). Slash was subjectively estimated on a scale from 1 (no slash) to 5 (a lot of slash).

Aspect

The aspect of the ground around each stump was recorded using a hand-held compass.

Slope

The slope of the ground around each stump was recorded using a hand-held clinometer.

Soil

The soil type of each site was determined from forest records provided by the appropriate forest companies.

The larval populations in each stump were sampled by removing the bark. Both *H. ater* and *H. ligniperda* breed in the cambium layer just beneath the bark. The bark was removed by levering off strips of bark using a large (50 mm wide) chisel. All larvae (all species of wood-boring larvae) and any other insects revealed were collected and stored in 70% ethanol.

All bark was removed from the top of the stump, down to the top of the main roots (below the soil surface). While *Hylastes* species are essentially root dwellers (Clark 1932b, Crowhurst 1969) they are found in all parts of the stump beneath the bark. Sampling larval populations in the roots would have been too labour intensive. It was felt that a representative sample of the relative populations of *H. ater* and *H. ligniperda* could be obtained from the area of stump sampled in this study.

Larvae were sorted in the laboratory. May (1993) describes the larvae of *H. ater* and *H. ligniperda*. While the larvae of the two species resemble each other, they are easily distinguished using larval head capsule features. The frons (upper portion of the head capsule) of *H. ligniperda* bear a pair of dark, low tubercles (small knob-like protuberances) paramedially (May 1993). The head capsule of the larvae of *H. ater* does not have tubercles. The tubercles on *H. ligniperda* may be seen using a 10x hand lens when studying later instar larvae. The larvae of other species collected were identified to family level.

Data were analysed using analyses of variance (ANOVA) to investigate differences in the numbers of *H. ater* and *H. ligniperda* larvae between sites, using the statistical package SAS (PROC GLM, Version 6.12 for Windows, SAS Institute 1996). Pairwise multiple comparisons were conducted using Duncan's multiple range tests to determine the nature of the differences detected by ANOVA.

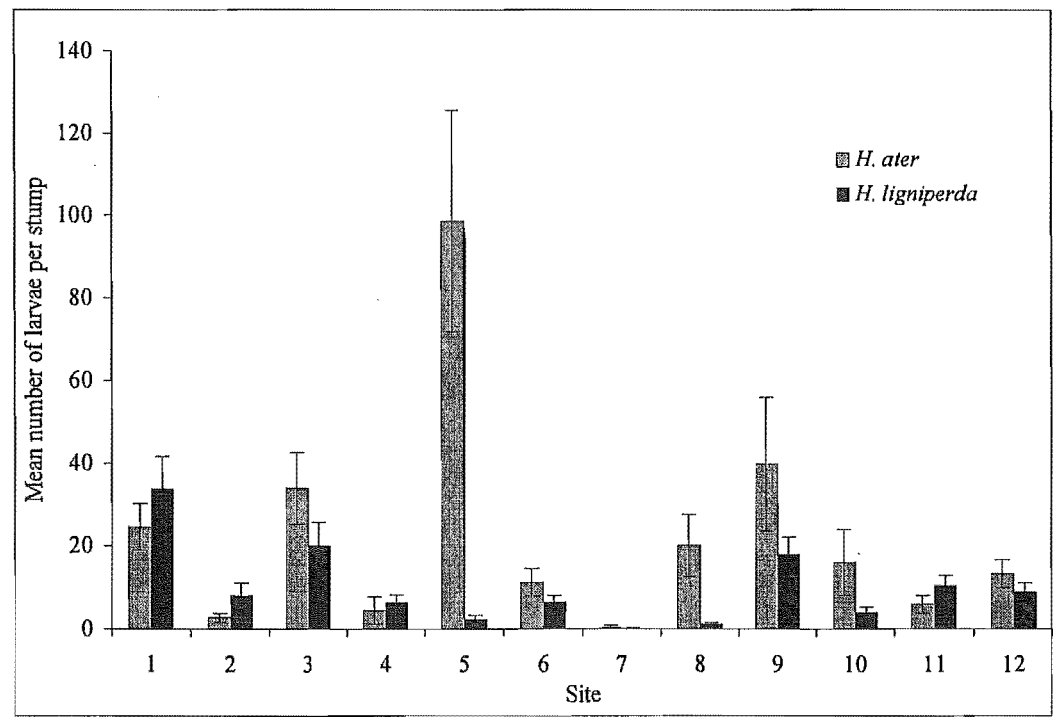
The dominant composition gradients for stump larval populations were investigated using the indirect ordination technique, Principal Components Analysis (PCA) (CANOCO

Version 2.1, ter Braak 1987). The associations between the first two PCA ordination axes data and the environmental variables were tested using the non-parametric technique of Spearmans Rank Correlation, using the statistical package SAS (PROC CORR, Version 6.12 for Windows, SAS Institute 1996).

2.1.3 Results

Larvae of *H. ater* and *H. ligniperda* were present in all sites (Figure 2.2). This indicates that the presence of one species did not result in the exclusion of the other. While *H. ater* dominated in four of the twelve sites, most sites had equal numbers of both species. The stumps in one site were dominated by *H. ligniperda*. Numbers of *H. ater* differed between sites ($F_{(11,228)} = 8.6$, $P < 0.001$). This was also true for *H. ligniperda*. Significant differences were found between sites ($F_{(11,228)} = 8.03$, $P < 0.001$). The high number of *H. ater* larvae in Site 5 is due to stumps in this site having large numbers of early instar larvae. The stumps in the other sites contained late instar larvae. Stumps in Site 5 had 'fresh' bark compared with stumps from the other sites. This indicates that Site 5 may have been harvested and colonised later in the year than the other sites. As the larval populations in these stumps developed, larval numbers probably would have declined due to 'normal' larval mortality processes. Larval numbers should have been similar to those from the other sites after a similar period of development.

Figure 2.2 Mean number of *H. ater* and *H. ligniperda* larvae found per stump in October 1997.



Principal component analysis (PCA) was used to explore the relationship between the stump larval populations. The PCA ordination of the stump larval populations indicates that there was much within site variation (Table 2.1, Figure 2.3). Overlapping sites on the axes indicates that the variation within sites was greater than the variation between the sites. Sites are not easily separated on either of the first two axes. With the exception of a few sites, most larval populations were similar. This is illustrated by the large cluster of stumps around the origin of the axes. The first axis (eigenvalue= 0.824) accounts for 82.4% of the variation in the data. The second axis (eigenvalue= 0.088) accounts for 8.8% of the variation in the data.

Figure 2.3 PCA site ordination of stump larval populations showing the relationship between sites (note each point is an individual stump).

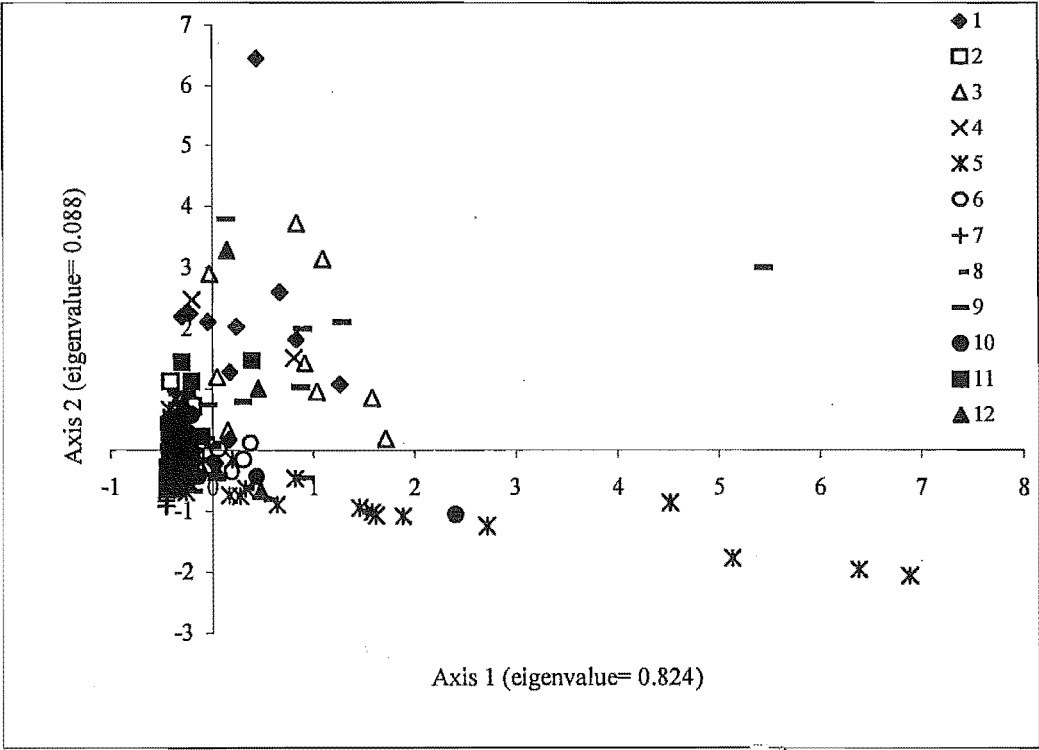


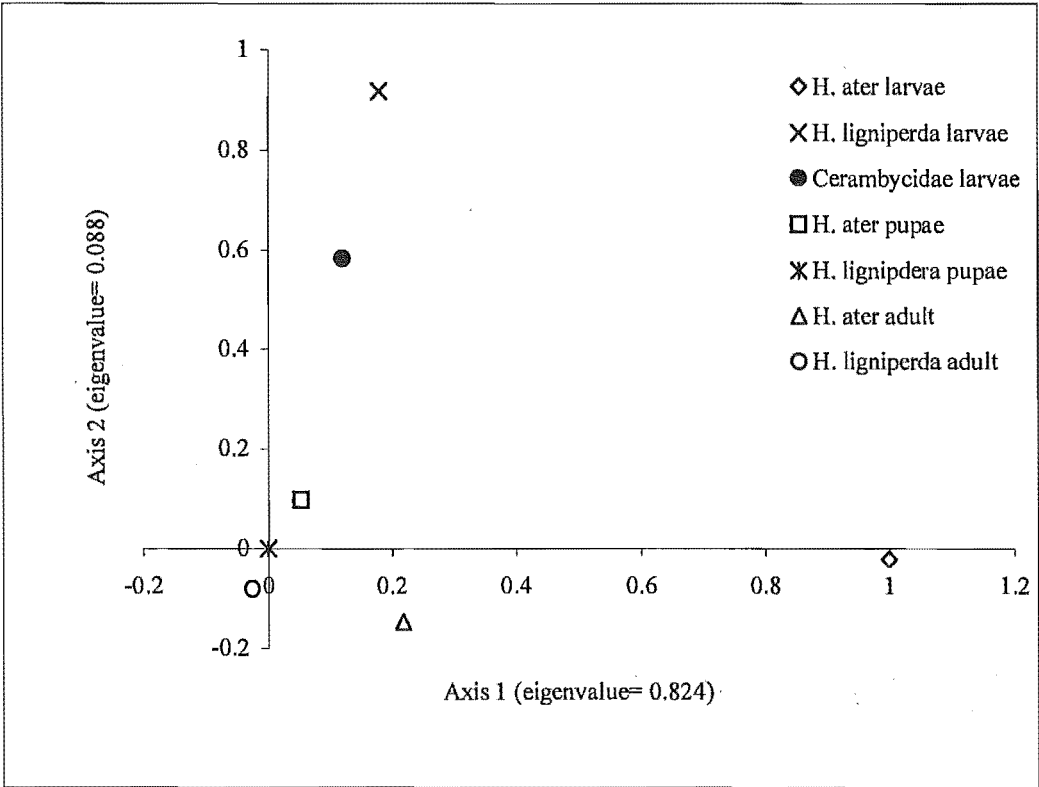
Table 2.1 Stump larval population PCA ordination summary.

Axes	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.824	0.088	0.046	0.039
Cumulative percentage variance of species data	82.4	91.2	95.8	99.7
Sum of all unconstrained eigenvalues	1.000			

The PCA ordination of the species data (Figure 2.4) indicates that *H. ater* and *H. ligniperda* larvae have the greatest influence on the spread of data across the first two axes in the site ordination. The position of each species relative to the origin of the axes indicates the relative strength and direction of its influence on the spread of the data on the site ordination. *H. ater* larvae are the most influential source of variation and account for most of the first axis variation. *H. ligniperda* has the greatest influence on the spread of data along the second axis. Site 5 shows the greatest spread across the first axes (Figure

2.3). This is due to the high number of *H. ater* larvae in a number of stumps (or the clumped distribution of the larvae in this site). This is also illustrated in Figure 2.2. Site 1 shows the greatest second axes spread (Figure 2.3), indicating that some stumps contained high numbers of *H. ligniperda* larvae (and cerambycid larvae).

Figure 2.4 PCA species ordination of stump larval populations indicating the relative strength and direction of species effects.



Spearman’s rank coefficients (Table 2.2) between the first two axes of the PCA site ordination and the environmental variables indicate that there were no significant correlations between the stump larval populations and the environmental variables measured. It is likely that other factors influenced stump larval populations. These were investigated in the following sections.

Table 2.2 Spearman's rank correlation coefficients calculated between the environmental variables and the first two PCA larval population ordination axes.

Environmental variables	Axis 1	Axis2
Outer bark area	0.134	0.179
Inner Bark condition	0.420	0.015
Slash	0.130	0.112
Aspect	-0.035	-0.159
Slope	-0.192	0.106
Soil type	-0.315	-0.172

2.1.4 Discussion

H. ater and *H. ligniperda* both occupied the same habitat during the winter months. One species did not exclude the other. This study investigated the overwintering larval populations. It was not known whether similar relationships would be observed during other months.

While relative numbers of larvae differed between sites, this was largely due to Site 5 having very high numbers of *H. ater* larvae. The relative differences in larval numbers both within and between sites were not related to the environmental variables measured. The PCA site ordination shows that there is much within site variation of stump larval populations.

It is possible that some of the environmental variables investigated in the study were of secondary influence (i.e. area of bark), affecting the survival of larval populations. While the relative numbers of colonising adults of both species were unknown, these could influence respective larval populations. Until relative numbers of adult colonisers of both species are assessed in relation to numbers of their offspring, it will not be possible to determine the factors that influence larval populations.

The main objective of this study was to investigate stump larval populations to develop a sampling programme to understand aspects of the biology and behaviour of *H. ater*. Clearly this cannot be undertaken without considering *H. ligniperda*. The effect that *H.*

ater and *H. ligniperda* may have on each other (conspecific competition), could not be assessed without data on adult colonisation. Therefore a sampling programme to investigate aspects of the ecology and behaviour of *H. ater* should consider the following:

- The relative number of *H. ater* and *H. ligniperda* individuals colonising sites
- Any potential interactions between adults and larvae of both *H. ater* and *H. ligniperda*
- Larval survival and factors that may influence it
- Any study should be over the duration of one year

2.2 A study investigating aspects of the ecology and behaviour of *H. ater* and *H. ligniperda*

The preliminary study (section 2.1) showed that both *H. ater* and *H. ligniperda* larvae were present in the same breeding material during winter months. Results indicated that to understand the behaviour of *H. ater*, it was important to consider the role of *H. ligniperda* in the forest system. This ecological study aimed to investigate the breeding and behavioural habits of the two species more closely. A better understanding of the two species would help when developing strategies that minimise the risk associated with *H. ater*.

Understanding the ecology and behaviour of *H. ater* required investigation into three areas that relate to its breeding habits. These areas encompassed annual activity (flight), colonisation and subsequent larval development, and adult emergence.

2.2.1 The annual flight activity and colonisation behaviour of *H. ater* and *H. ligniperda*

2.2.1.1 Introduction

The flight activity of *H. ater* and *H. ligniperda* may give indications as to how the two species interact with each other. Adult flight represents the period during which new adults emerge from brood material, search for and colonise new breeding material to establish future generations. Monitoring the flight activity of both species meant it was possible to identify differences in the periods of activity since differences in the colonisation or host-searching activity provide important clues to help understand the behaviour and development of both species. This may be valuable when identifying where *H. ater* might be a risk to forestry.

The objectives of this study were to:

- Determine whether the adults of both species were active all year round
- Investigate whether either species had a period of enhanced adult activity
- If peak periods of adult activity did exist in both species, determine whether these were synchronised or occurred at separate times of the year
- Investigate whether periods of peak activity were influenced by climatic variables or any other environmental factors
- Determine whether any periods of peak activity occurred at a similar time each year
- Investigate whether sites harvested at the same time were colonised by equal numbers of adult beetles
- If levels of colonisation differed with respect to site, investigate factors that may influence colonisation

2.2.1.2 Methods

Twenty-eight sites were selected in second rotation *P. radiata* forests (Kinleith and Kaingaroa Forests) in March 1998. Sites were selected during this period as flight activity was expected.

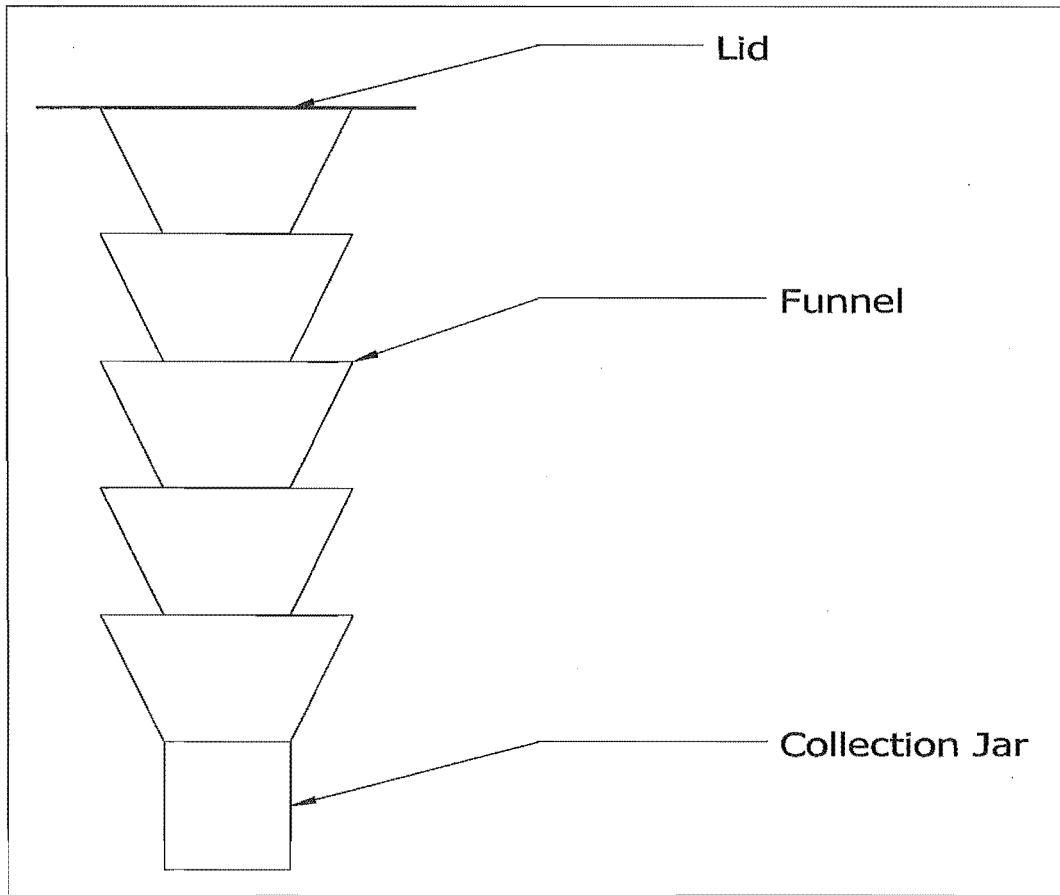
Sites were chosen that had been harvested within two weeks prior to selection. Information on the harvesting activity in individual sites was provided by Carter Holt Harvey Forests and Fletcher Challenge Forests, and by the harvesting crews operating in the forest.

A variety of sites were selected over a wide geographical area in both forests. The distance to travel around the sites was approximately 200 km.

The relative colonisation activity (flight activity) in each site was assessed using Lindgren funnel traps (Lindgren 1983). Five-funnel traps were constructed by suspending large diameter (21 cm) funnels above each other using 2 mm cord (Figure 2.5). The distance between each funnel was 10 cm. The top funnel of each trap was covered with a 21 cm diameter plastic plate to prevent debris from entering the trap. A plastic screw-top collection jar with a gauze covered drainage hole at the bottom was attached to the bottom funnel. Each trap was suspended from a 1.8m wooden stake. The traps were sturdy in construction and able to withstand the elements (i.e. rain and wind) for long periods of time.

A 100 ml glass test-tube was placed in each plastic collection jar. Each test-tube contained 80 ml of raw turpentine as an attractant. Raw turpentine is an attractant for *Hylastes* species (Gara & Vité 1962, Löytyniemi *et al* 1988b, Phillips 1990) and was available at no cost. The raw turpentine was a product of chemical pulping. The monoterpenes that make up the raw turpentine were distilled from chemical pulp digesters (Burdon *et al* 1992a). The raw turpentine used in each trapping season came from one batch.

Figure 2.5 Construction of five-funnel Lindgren type funnel trap.



An assumption was made that the raw turpentine would attract both *H. ater* and *H. ligniperda*, and that the attractiveness would be the same for both species. The attractiveness of the raw turpentine was tested with a number of other potential attractants during the later stages of this project. The results of these trials, presented in detail in Chapter Six, showed that this raw turpentine was not attractive to either *H. ater* or *H. ligniperda*. Therefore, any *H. ater* or *H. ligniperda* individuals caught during this study were due to accidental catches, or visual attraction to the traps. While more individuals would have been caught if a more effective attractant had been used, the number of *H. ater* and *H. ligniperda* caught represented the relative activity of each species in each site.

Two traps were erected in each site, approximately 80m apart. Traps were located 30m into each site to avoid any potential edge effects.

Traps were serviced fortnightly from 18 March to 11 May 1998. Each service consisted of removing all insects caught and storing them in 70% ethanol solution. The turpentine was replaced during each trap service.

Each trap catch was sorted and counted in the laboratory. Only those species directly relevant to this study were counted. A third species of bark beetle, *Pachycotes peregrinus*, was occasionally intercepted. *P. peregrinus* is a native species that occurs throughout New Zealand and attacks the wood of many native and exotic softwoods (Bain 1977b, May 1993). This species was not included in any analyses, as it was rare.

At the end of the trapping period, traps were removed from the forest and cleaned in preparation for the following trapping season. Traps were left in some sites to monitor flight activity during winter months. These traps were serviced once each month during winter.

In October 1998 (spring) 19 new sites were selected. Sites were selected to include a variety of site types, and were spread across the two forest estates.

The selection of sites harvested prior to March 1998 was based on the harvesting history of each site provided by harvesting crews and forest staff. Following the assessment of larval populations in these sites (section 2.2.2) it was felt that site selection based on harvesting activity should be made with greater precision. Traps were positioned in areas that had been harvested the previous day where possible. This caused some difficulty as harvesting operations often continued in the immediate area. While this activity was unlikely to influence the results of the study, access was made more difficult on occasions. Sites were not selected if traps could not be placed in an area that was harvested within one week of the traps being erected.

While traps were serviced fortnightly in autumn of 1998, more accurate data could be obtained if traps were serviced weekly. Weekly collections were made over a seven-month period from 1 October 1998 to 5 May 1999.

It was important to assess larval populations from sites that were colonised at different times throughout the summer period. Two further groups of sites were added to the

trapping programme during the summer period. Four sites were selected and monitored in January 1999. A further nine sites were added in March 1999 giving a total of 32 sites.

Weekly trap collection in all sites continued until the final collection was made on 5 May 1999. At the end of the trapping period, traps were removed from the forest and cleaned in preparation for the following trapping season. Traps were left in some sites to monitor flight activity during winter months. These traps were serviced once per month during winter.

In October 1999, 17 new sites were selected. As with the previous summer, three additional sites were selected later in the summer period (March 2000) and were added to the trapping programme.

Unlike the previous trapping programme, traps were serviced at fortnightly intervals. While there was an associated loss in the sensitivity of the data collected, the large effort required for weekly servicing over such a long period was not possible during the 1999/2000 trapping period.

The National Institute of Water and Atmosphere supplied climatic data for the study area during the period of study. These data were collected at the Rotorua Airport climate station (B86133). While there was likely to be variation in climate between the Rotorua Airport and the sites in this study, it was not possible to collect climate data within the forest estates. The data were used to investigate the influence of relative changes in climate on flight activity over time.

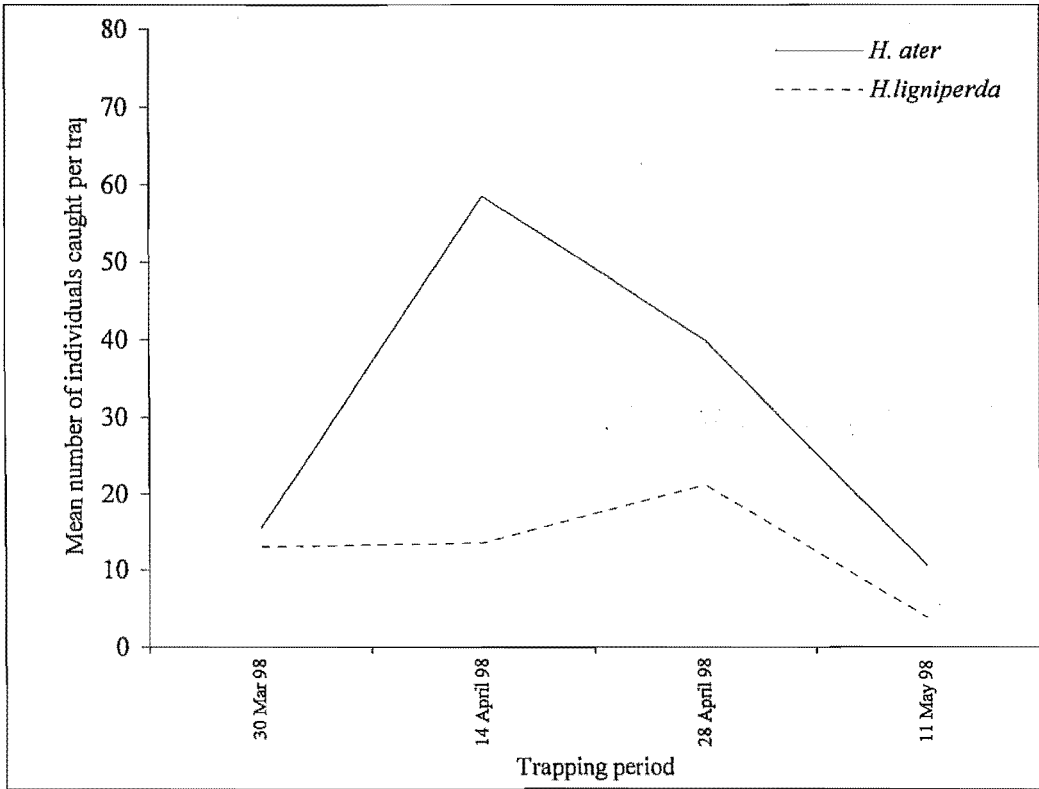
Data were investigated using analyses of variance (ANOVA) to detect differences in the colonisation of *H. ater* and *H. ligniperda* between sites (PROC GLM, Version 6.12 for Windows, SAS Institute 1996). Pairwise multiple comparisons were conducted using Duncan's multiple range tests to determine the nature of the differences detected by ANOVA.

2.2.1.3 Results and Discussion

Trapping period 18 March to 11 May 1998

Flight activity during autumn was dominated by *H. ater* (Figure 2.6). The activity of *H. ater* increased sharply at the end of March and peaked during mid-April. The mean number of *H. ligniperda* individuals caught was reasonably constant throughout the period before dropping at the end of the trapping period. During the winter months both *H. ater* and *H. ligniperda* were trapped, however numbers were minimal. There were not any periods of substantial activity during winter. The results of the catches during the winter period are not presented here.

Figure 2.6 Mean number of *H. ater* and *H. ligniperda* individuals caught per trap during the period 18 March to 11 May 1988.



Trapping period 1 October 1998 to 5 May 1999

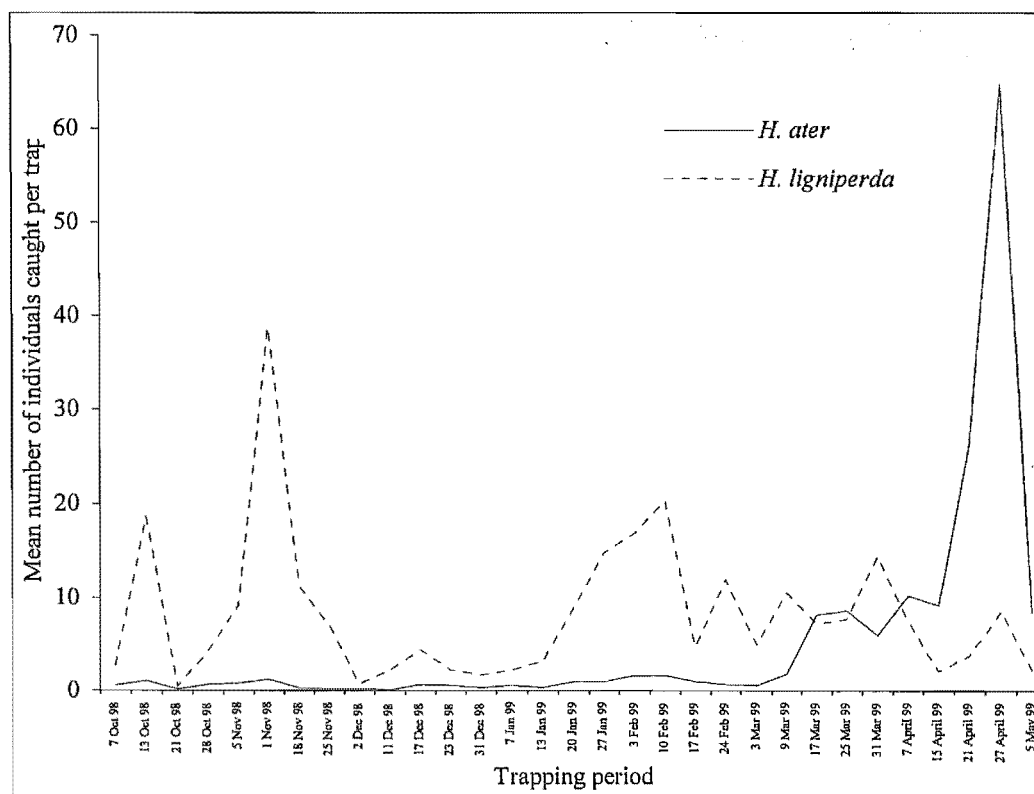
Flight activity of *H. ater* and *H. ligniperda* fluctuated over the seven-month trapping period (Figure 2.7). A peak of flight activity during spring and early summer (7 October to mid-November) was dominated by *H. ligniperda*. This initial activity was followed by a month of relatively low activity for both species. A second period of increased activity by

H. ligniperda began during January 1999 and continued for approximately three months. This period of activity by *H. ligniperda* did not peak as sharply as observed during spring. However, it did continue for a longer time. *H. ligniperda* flight activity dropped away at the end of summer/autumn.

During most of the spring and summer months, the flight activity of *H. ater* was very low in comparison to *H. ligniperda* (Figure 2.7). *H. ater* adults were found during the year. Slight increases in activity, which coincided with *H. ligniperda* flight, may be due to *H. ater* responding to similar cues (e.g. climate).

At the end of the 1998/99 summer period the activity of *H. ater* increased substantially (during March) before sharply peaking in the latter part of April. This sharp peak in flight activity lasted for a short time (2-3 weeks) in comparison with the *H. ligniperda* peaks of activity. This peak in *H. ater* activity was more intense than any of the two *H. ligniperda* periods of activity. Following this, the mean numbers of both species caught fell dramatically.

Figure 2.7 Mean number of *H. ater* and *H. ligniperda* individuals caught per trap during the period 1 October 1998 to 5 May 1999.



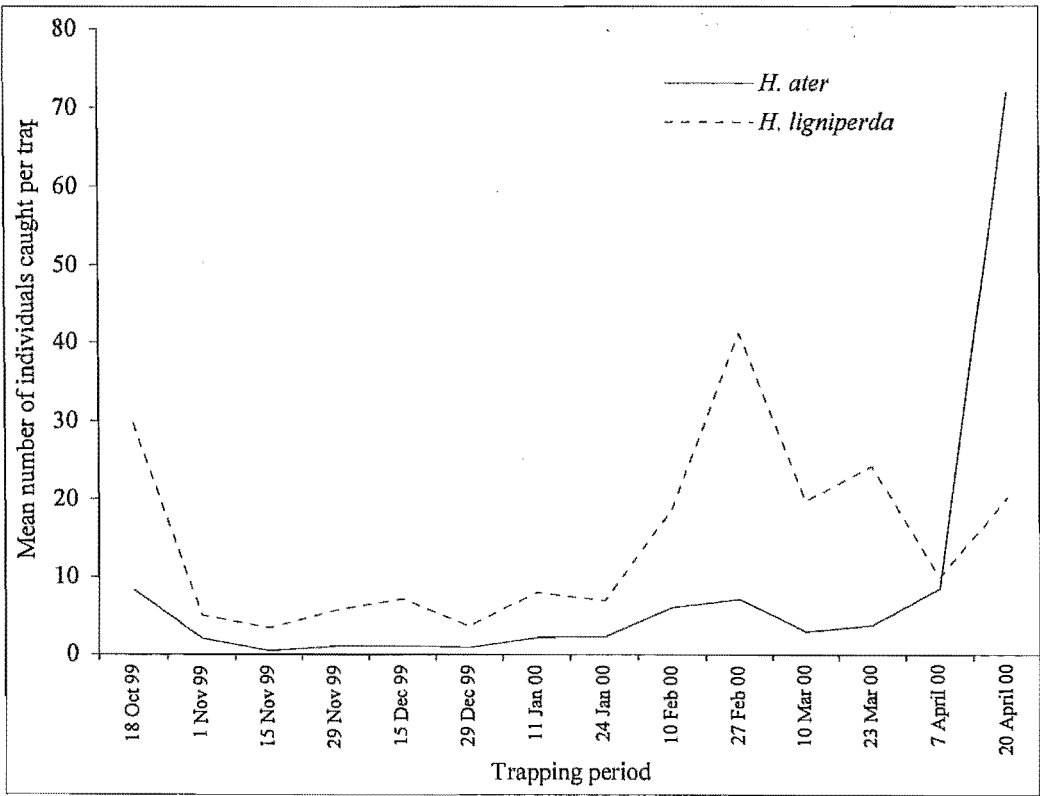
Trapping period 4 October 1999 to 20 April 2000

The length of time between trap servicing was extended from one week (during 1 October 1998 to 5 May 1999) to two weeks during this trapping period.

Flight activity during this period was consistent with the activity of both species during the previous summer (Figure 2.8). The activity of *H. ligniperda* peaked in spring as with the previous year. There was a greater amount of *H. ater* activity at this time compared with the previous year. However, the spring period was still dominated by *H. ligniperda*. Following this initial activity, the numbers of both species fell to relatively low levels. At the end of January there was a marked increase in *H. ligniperda*, which peaked at the end of February before falling away during April.

The flight activity of *H. ater* increased slightly at the end of January 2000, before increasing sharply during April. This sharp increase in activity was consistent with the previous summer. *H. ater* activity at this time was (as with the previous summer) more intense compared with any period of *H. ligniperda* activity.

Figure 2.8 Mean number of *H. ater* and *H. ligniperda* individuals caught per trap during the period 4 October 1999 to 20 April 2000.



Trapping was discontinued after 20 April 2000. It was not possible to determine whether the last trapping period was the period of greatest *H. ater* activity. However, given the consistency of activity between the two summers, it is likely that the activity of both species fell soon after the trapping programme was completed.

The activity of each species was reasonably consistent between the two years. The periods of peak activity of *H. ater* and *H. ligniperda* were at different times. The flight activity of *H. ater* was characterised by one dominant period of flight just before winter. This indicates that *H. ater* may be univoltine in New Zealand. This period of *H. ater* activity was more intense compared with periods of *H. ligniperda* activity. Outside of this period of activity, populations of active adult *H. ater* were low.

The flight activity of *H. ligniperda* during the summer periods was characterised by two periods of peak activity. Therefore, *H. ligniperda* may be bivoltine in New Zealand. The first period of *H. ligniperda* activity was in spring. The second period occurred from January to March. The dominant periods of adult flight activity of the two species were separated in time, although they inhabited the same stumps. Possible explanations and the implications of this are discussed further below.

It is possible to suggest hypotheses regarding the adult flight activity and the relationship of this activity to the lifecycle of the two species. The flight activity of the two species represents a search for new breeding (and food) material. It is during these periods of flight that new sites are colonised and new generations are established. The *H. ligniperda* adults trapped in spring were most likely to have overwintered as late instar larvae or as adults. The second period of peak *H. ligniperda* activity was most likely to be the result of oviposition during spring colonisation.

Subsequent generations resulting from *H. ligniperda* colonisation and breeding during the mid-summer period may develop through to maturity before the end of summer. These adult beetles may emerge and colonise habitat before the end of summer, or perhaps overwinter as adults. The offspring produced by colonising adults during the later part of the second activity period of *H. ligniperda*, would have been unable to develop fully before winter. These individuals would have overwintered as late instar larvae and completed their development before the spring emergence.

In contrast, a period of emergence and the colonisation of new habitats at the end of summer dominated the breeding and development activity of *H. ater*. The offspring produced by these adults would have overwintered as eggs and early instar larvae. These larvae continued development during the following summer and their emergence coincided with the period of peak *H. ater* activity at the end of that summer.

While both the lifecycles of *H. ater* and *H. ligniperda* were characterised by periods of dominant activity, individuals colonised new breeding habitats and bred outside of these periods. This was illustrated by the presence of both species at low levels year round. The periods of dominant flight activity indicate that *H. ligniperda* is bivoltine and *H. ater* is univoltine in New Zealand.

The influence of climate on the flight activity of individuals

Potential effects of temperature and atmospheric pressure were investigated using the flight activity data from the 1998/99 period. The data collected during the other trapping periods (collected at fortnightly intervals) was not sensitive enough to reflect effects of climate on the flight activity of *H. ater* and *H. ligniperda*. The data from weekly collections was not sensitive enough to test the climatic variables that influence flight activity. However, the relationships identified provided information for further work in this area.

H. ligniperda activity appeared to be influenced by atmospheric pressure peaks (Figure 2.9). The three largest peaks in *H. ligniperda* flight activity corresponded with the three most significant peaks of mean weekly atmospheric pressure (Figure 2.9). Mean weekly temperature was not clearly related to *H. ligniperda* flight (Figure 2.10). Mean weekly temperature was higher during the mid-summer months.

A relationship between *H. ater* flight activity and atmospheric pressure was not apparent during the 1998/99 summer period (Figure 2.9). This could have been a function of the low levels of *H. ater* activity during this period. The drop in temperature and/or atmospheric pressure may have triggered the peak of *H. ater* flight activity during the latter part of April 1999.

Figure 2.9 Mean weekly catch of *H. ater* and *H. ligniperda* individuals from 1 October 1998 to 5 May 1999, with mean weekly atmospheric pressure during the same period.

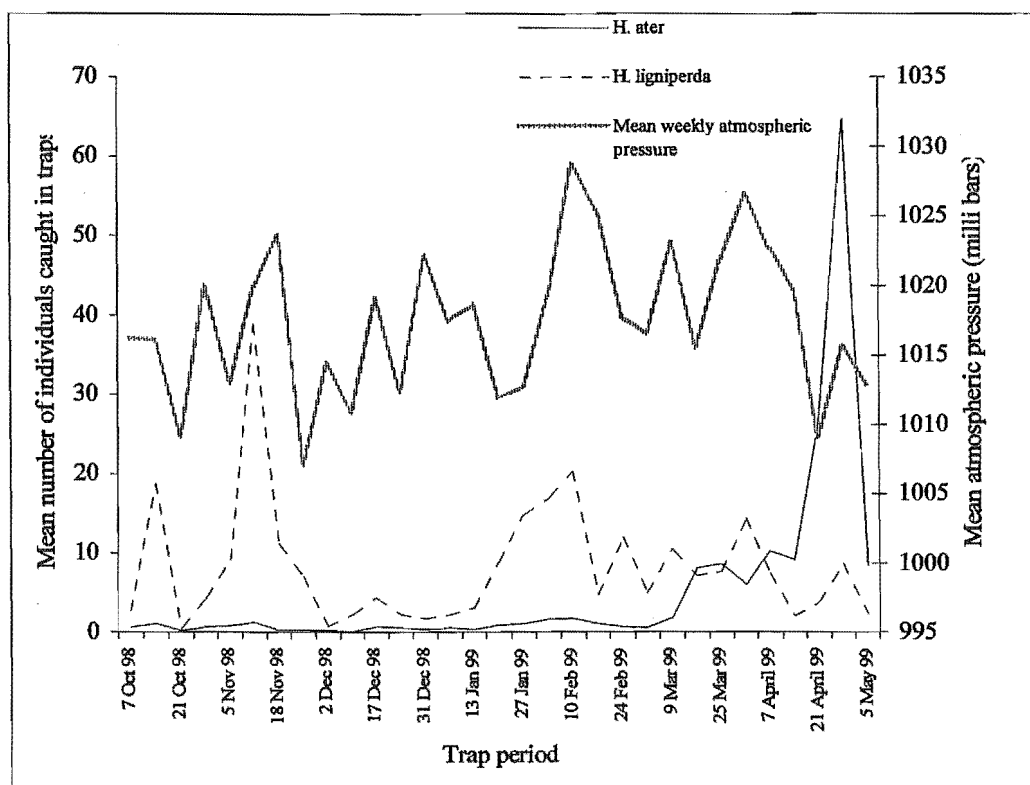
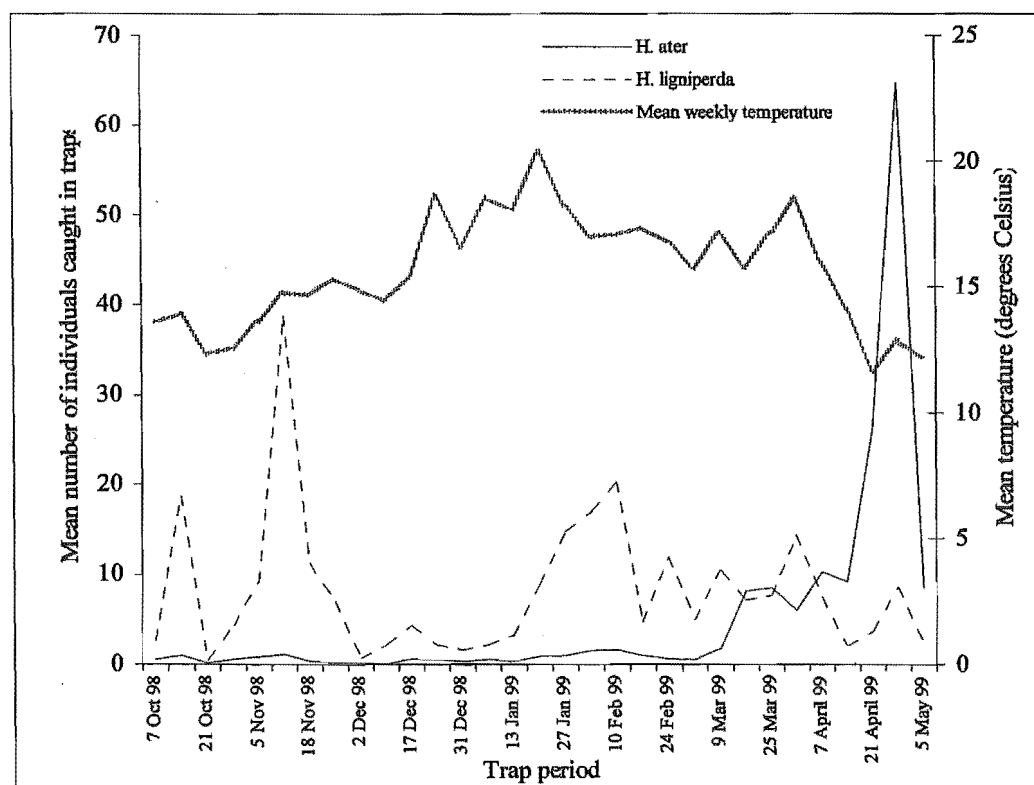


Figure 2.10 Mean weekly catch of *H. ater* and *H. ligniperda* individuals from 1 October 1998 to 5 May 1999, with mean weekly temperature during the same period.



During the 1998/99 summer trapping period three mass flights were observed in the forest. On the first occasion, a large flight of *H. ligniperda* was witnessed. This flight occurred during mid-afternoon on a still warm day during the middle of November 1998. An enormous number of *H. ligniperda* individuals could be observed over a distance of approximately 200m.

The second mass flight of *H. ligniperda* was witnessed in similar conditions to those described above. It occurred during the mid-afternoon in late January 1999 and probably involved fewer individuals. However, on this occasion there did not appear to be as many individuals flying.

The final occasion when this phenomenon was witnessed was on 27 April 1999. During the mid-morning, large numbers of *H. ater* were observed being blown in a southerly direction by a light northerly breeze. Aside from the light wind, the day was warm and the sky was clear. *H. ater* individuals could be seen flying over several hundred metres, from near ground level to approximately 40m above the ground. During the late afternoon on the same day, a similar flight of *H. ater* was observed approximately 30 km from the first flight. The temperature and wind strength were similar to that observed earlier in the day. However, there were many more beetles flying. Their flight during this time was in many cases rather haphazard, and beetles flying low to the ground were observed colliding with stumps and myself.

All the areas where the flights were observed were in recently harvested areas which beetles were colonising. On all occasions the weather was still and clear, although a light northerly breeze was blowing on the final occasion. Similar flights were observed during the course of this study by harvesting crews. However, it was not possible to determine what species of beetle was involved.

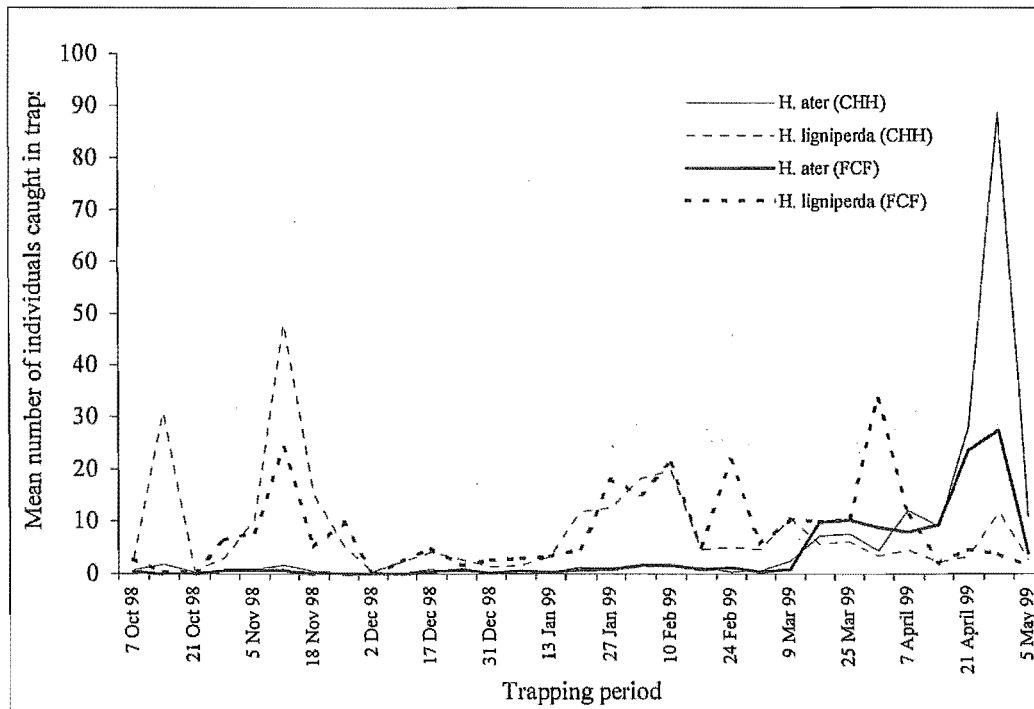
While mass flights are known for Scolytids, they are not often observed. The factors that influence mass flight activity are not thoroughly described. Lindelöw (1992) observed flight activity of *Hylastes cunicularis* in Germany when spring temperatures were above 20 °c. *Hylastes nigrinus* (Mann.) is reported to disperse by flight during brief periods of 'favourable' weather (Zethner-Møller & Rudinsky 1967). Rieseke and Raffa (1990) suggest that *Hylobius pales* flight is strongly influenced by weather patterns. *Dendroctonus valens*

flight activity in Blodgett Forest (California) was confined to warmer periods, with beetles requiring a minimum temperature of 20°C before flying (Hobson 1992). Flight activity appeared to decrease at temperatures above 29.5°C (Hobson 1992). Daily flight activity varied depending on wind strength and the temperature of the previous day (Hobson 1992).

Factors that may influence the number of beetles colonising different sites

Comparisons of the Kinleith and Kaingaroa forests (Figure 2.11) indicate that periods of peak flight activity were synchronised between the two forests from 1 October 1998 to 5 May 1999. Individuals over large areas may be responding to similar cues.

Figure 2.11 Mean weekly trap catches of *H. ater* and *H. ligniperda* adults in FCF and CHH forest estates during the summer of 1998/99.



The period of site colonisation was defined as the two-month period of flight activity following the harvesting of a site. After two months most stumps would have been unsuitable for colonisation, as they contained breeding populations. While some individuals may have established broods in previously colonised stumps, observations made during the sampling of larval populations (section 2.12) indicated that this was

uncommon. Competition by established and developing broods would probably effect the development of the later broods.

The colonisation activity of *H. ater* and *H. ligniperda* was assessed for the four periods of flight activity to determine whether the flight activity of each species differed between sites (Table 2.3). Subsequent larval populations were sampled following this colonisation (refer to section 2.2.2). Data from recently harvested sites were used to assess the colonisation activity in different sites. The number of individuals colonising sites harvested during the different time periods was different for both species between sites (Table 2.3).

Table 2.3 The analysis of numbers of individuals colonising sites shows that there was significant variation between sites for each species.

Sites harvested during the period	<i>H. ater</i>	<i>H. ligniperda</i>
18 March 1998 to 11 May 1998	$F_{(27,182)} = 1.81, P < 0.05$	$F_{(27,182)} = 2.6, P < 0.001$
1 October 1998 to 25 November 1998	$F_{(18,258)} = 2.89, P < 0.001$	$F_{(18,258)} = 2.82, P < 0.001$
13 January 1999 to 3 March 1999	$F_{(3,60)} = 2.68, P > 0.05^*$	$F_{(3,60)} = 1.73, P > 0.05^*$
17 March 1999 to 5 May 1999	$F_{(8,104)} = 4.01, P < 0.001$	$F_{(8,104)} = 3.04, P < 0.01$

* the number of colonising beetles did not differ between sites

Some sites were more attractive to colonising beetles. A number of factors may influence the attractiveness of a site. The harvested area and the amount of harvesting in close proximity are likely to effect the relative level of volatiles in the immediate area. Stumps in different sites may release qualitatively different volatiles (Thorin & Nömmik 1974, Yazdani & Nilsson 1986, Tilles *et al* 1986a,b). This may be due to genetic differences (Löyhtyniemi & Hiltunen 1976), different growing conditions, site types (i.e. soil type) or microclimate effects that may influence how volatiles are dispersed into the air.

The distance between a newly harvested site and an emerging beetle population will influence the number of individuals that may reach a site (De Jong & Sabelis 1988). Following emergence, adult beetles are more likely to colonise nearby breeding material. The greater the distance travelled the more diluted a population becomes (De Jong & Sabelis 1988).

The main points that arose from this study were as follows:

- Both *H. ater* and *H. ligniperda* have peak periods of flight activity
- These periods of activity are synchronised within species
- Outside of these periods adults are active, however at much reduced levels
- Synchrony with regard to the colonisation of habitat and breeding is most likely to ensure mate finding
- *H. ater* is univoltine and *H. ligniperda* is bivoltine in New Zealand
- The initiation of mass flight in *H. ater* may have been related to a combination of temperature and atmospheric pressure
- The number of colonising *H. ater* and *H. ligniperda* colonising sites differs between sites. A number of factors may effect the attractiveness of a site to both species

2.2.2 The larval composition of stump populations

2.2.2.1 Introduction

The preliminary investigation of stump larval populations showed that during winter broods of *H. ater* and *H. ligniperda* occupied the same stumps. The study of the flight activity of *H. ater* and *H. ligniperda* (section 2.2.1) indicated that peaks of flight activity of the two species were separated in time. As *H. ligniperda* was the most active species during spring and most of summer, it was expected that larvae of this species would dominate sites harvested during this period. As *H. ater* was very active during the final part of summer, *H. ater* larvae should have dominated sites harvested during this period.

In addition to investigating the relationship between larval populations and the flight activity of colonising adults, efforts were made to identify site characteristics that enhanced the survival of larvae in particular sites.

The objectives of this study were to:

- Compare the species composition of stump larval populations in different sites
- Investigate whether there was a relationship between colonisation activity and the resulting larval populations in a site

- Determine whether the species composition of stump larval populations at different times of year reflected differences between *H. ater* and *H. ligniperda* adult flight activity
- Investigate whether differences in larval populations (or larval survival) were related to site factors

2.2.2.2 Methods

Stump larval populations were sampled from sites colonised during four of the periods of colonisation activity described above in section 2.2.1. Larval populations were sampled from selected sites harvested during (i) autumn 1998 (ii) spring 1999 (iii) mid-summer 1998/99, and (iv) autumn 1999.

Between four and seven sites were randomly selected from sites harvested during each of the periods above (Appendix 1). Sites harvested during the spring and mid-summer periods were sampled approximately three months following harvesting. Sites harvested at the end of summer were sampled approximately seven months after harvesting. This ensured that a reasonable amount of larval development had occurred and larvae were more easily collected and identified. Distinguishing between the two species (*H. ater* and *H. ligniperda*) with later instar larvae was less difficult than with early instar larvae.

The larval populations and site factors in each site were sampled using the methods described in section 2.1.2.

2.2.2.3 Results and Discussion

The stump larval populations (Figure 2.12 to 2.15) reflected the colonisation activity of both species during the period following harvesting (Figures 2.6 & 2.7).

Figures 2.12 to 2.15 show the mean number of *H. ater* and *H. ligniperda* larvae found in stumps in each site. These larval populations reflect the colonisation patterns of the site. Sites harvested when *H. ligniperda* dominated the colonisation (sites colonised 1 October

to 25 November 1998 (Figure 2.13) and 13 January to 3 March 1999 (Figure 2.14)) were dominated by *H. ligniperda* in stump larval populations.

Sites harvested at the end of summer/autumn were characterised by *H. ater* colonisation activity. The larval populations of sites harvested during this period were, in general, dominated by *H. ater* (sites colonised from 18 March to 11 May 1998 (Figure 2.12) and 17 March to 5 May 1999 (Figure 2.15)). However, there were some inconsistencies for the period colonised from 18 March to 11 May 1998 (Figure 2.12). While these sites were dominated by *H. ater* colonisation, stumps in some sites (e.g. 14, 16, 18, 19) had relatively high numbers of *H. ligniperda* larvae. It was likely that these sites were harvested considerably earlier than the date recorded. If so, then the larval populations in these sites would be more likely to reflect the activity of colonising beetles prior to the initiation of the trapping programme. *H. ligniperda* was most likely to be active prior to the beginning of the trapping programme (section 2.2.1). The relatively high numbers of *H. ater* larvae in Sites 13 and 17 in comparison to *H. ligniperda* larvae reflect the dominant colonisation of *H. ater* adults during the period following harvesting, and conform with the pattern described above.

Figure 2.12 Mean number of *H. ater* and *H. ligniperda* larvae in stumps from sites colonised from 18 March to 11 May 1998.

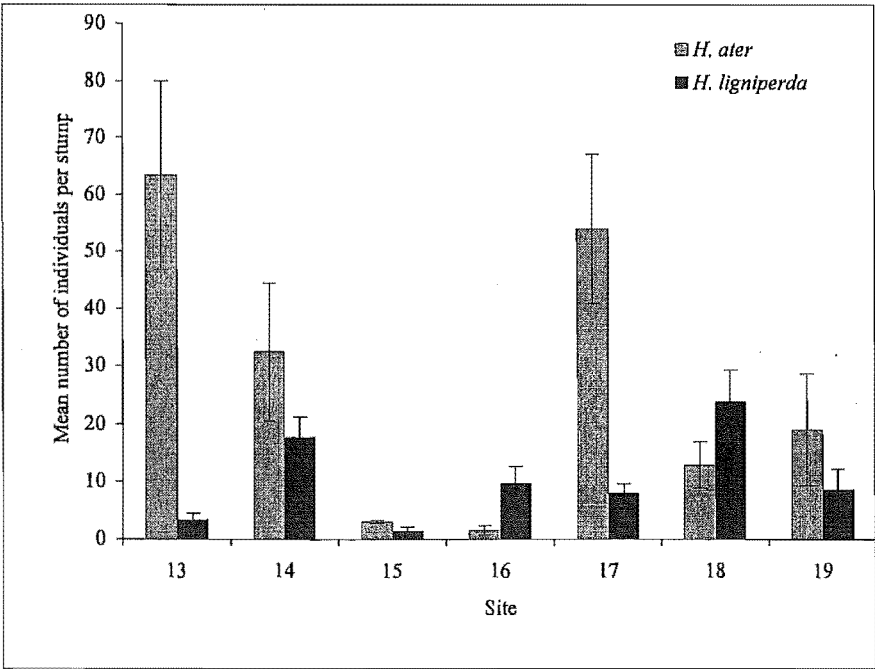


Figure 2.13 Mean number of *H. ater* and *H. ligniperda* larvae in stumps from sites colonised from 1 October to 25 November 1998.

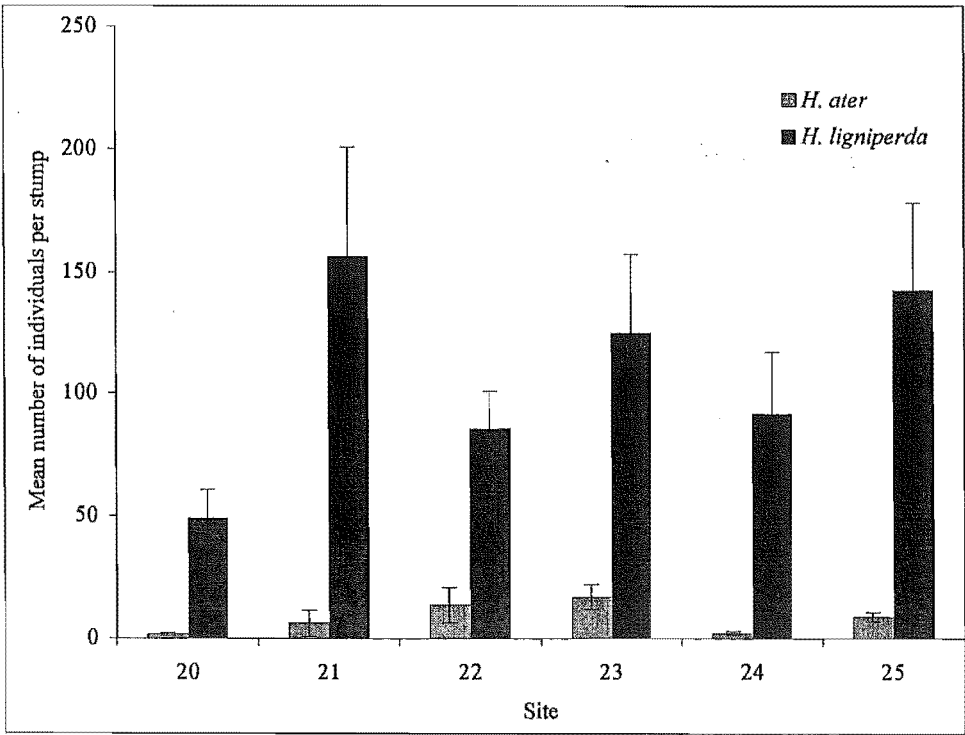


Figure 2.14 Mean number of *H. ater* and *H. ligniperda* larvae in stumps from sites colonised from 13 January to 3 March 1999.

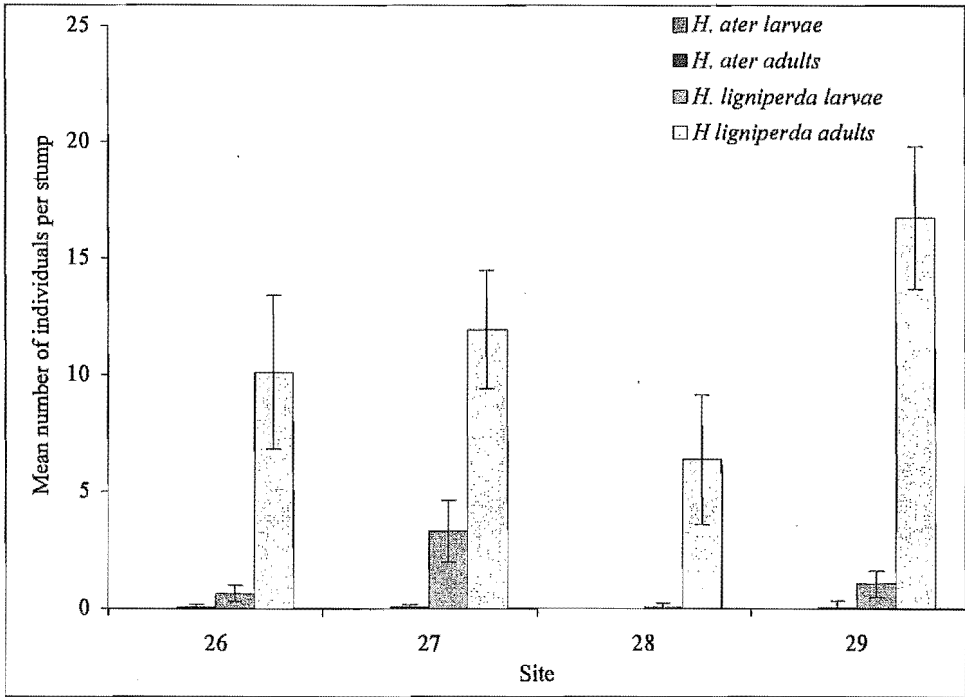
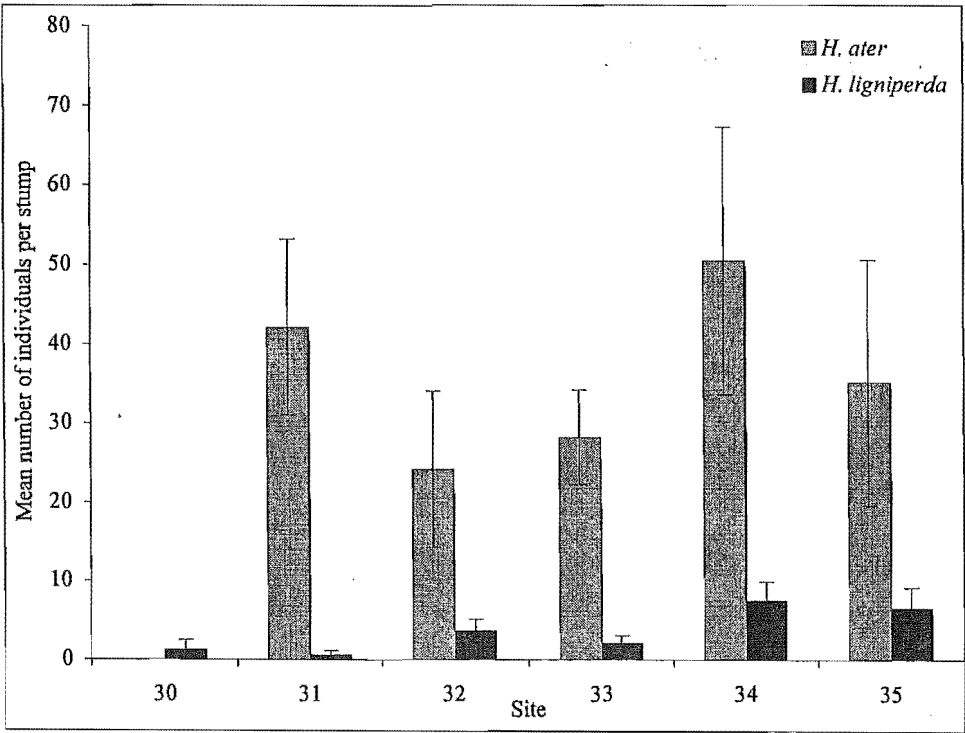


Figure 2.15 Mean number of *H. ater* and *H. ligniperda* larvae in stumps from sites colonised from 17 March to 5 May 1999.



The PCA ordination plots of stump populations from sites harvested prior to each colonisation period illustrate the relationship between adult flight activity and subsequent larval populations (Figures 2.16 to 2.23).

The site ordinations (Figures 2.16, 2.18, 2.20, 2.22) show that larval populations from sites colonised during the same period were similar. The first two ordination axes account for most of the variation in the data (Tables 2.4 to 2.7). The site ordinations indicate that the variation of stump populations within sites was greater than the variation of populations between sites. The PCA ordinations of the species data (Figures 2.17, 2.19, 2.21, 2.23) show the strength and direction effects of the species data on the site ordinations. The relative distance and direction of each species from the origin of the axes indicates the relative strength and direction of its effect. The ordination eigenvalues in Tables 2.4 to 2.7 show the amount of variation in the data that each ordination axis explains.

The PCA ordination scatter (Figure 2.16) of sites colonised during the period from 18 March to 11 May 1998 shows that stumps had similar larval populations. The ordination indicates that the variation of populations within sites was greater than between sites, except for a few stumps in Sites 13, 16 and 17. The species ordination indicates that *H. ater* larvae were the strongest influence on the scatter of stumps across the first axis, while *H. ligniperda* adults had the greatest influence across the second axis (Figure 2.17). Stumps (from Sites 13 and 17) at the right hand side of the first axis were dominated by *H. ater* larvae. Those stumps high on the second axis (site 16) contained more *H. ligniperda* adults in comparison to the other stumps sampled.

The PCA ordination scatter (Figure 2.18) of sites colonised from 1 October to 25 November 1998 shows that it was not possible to separate sites on the basis of stump larval populations. The PCA ordination scatter (Figure 2.19) of species data shows that *H. ligniperda* larvae had the greatest influence on the scatter of stumps across the first axis, while *H. ater* larvae were the strongest influence across the second axis. Stumps at the right hand side of the first axis had greater numbers of *H. ligniperda* larvae compared with the other stumps. Stumps high on the second axis contained more *H. ater* larvae. The high eigenvalue of the first axis indicates that differences in numbers of *H. ligniperda* larvae were responsible for most of the variation in the ordination.

The PCA ordination scatter (Figure 2.20) of sites colonised from 13 January to 3 March 1999 shows that the populations of most stumps were similar. However there is a reasonable spread across the axes in comparison to the site ordinations from the other time periods. The PCA ordination scatter (Figure 2.21) shows that *H. ligniperda* adults have the greatest influence of scatter on the stumps across the first axis while *H. ligniperda* larvae have the most influence on the second axis spread. This indicates that those stumps at the right hand side of the first axis had greater numbers of *H. ligniperda* adults compared with the other stumps. The presence of *H. ligniperda* indicates populations were more developed in these sites at the time of sampling. *H. ligniperda* adults were responsible for most of the variation in the ordination.

The PCA ordination scatter (Figure 2.22) of sites colonised from 17 March to 5 May 1999 shows that the larval populations of most stumps were similar to each. Much of the spread across the ordination, or variation within the sites, is along either the first or second axes. This indicates that species effects associated with each axis were the dominant sources of variations within the sites. The PCA ordination scatter (Figure 2.23) of the species data shows that *H. ater* larvae have the most influence on the scatter of stumps across the first axis while *H. ligniperda* larvae have the greatest influence across the second axis. The adult and pupal forms of *H. ater* and *H. ligniperda* and other species found in stumps are situated close to the origin on the species ordination, indicating that they had little influence with respect to the overall variation within the data. Site 30 had no *H. ater* larvae (Figure 2.15). *H. ater* adults did not successfully colonise this site. The presence of *H. ligniperda* adults and very few *H. ligniperda* larvae indicates that Site 30 was colonised by *H. ligniperda* just prior to the sampling of the larval populations, during the period of flight activity in spring (October to November) 1999. Sites that were not colonised during a period of flight activity may remain as suitable breeding material for some time, in this case approximately seven months.

Table 2.4 Stump larval population PCA ordination summary for sites colonised from 18 March to 11 May 1998.

Axes	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.754	0.159	0.073	0.012
Cumulative percentage (%) variance of species data	75.4	91.3	98.6	99.8
Sum of all unconstrained eigenvalues	1.000			

Table 2.5 Stump larval population PCA ordination summary for sites colonised from 1 October to 25 November 1998.

Axes	Axis 1	Axis 2	Axis 3	Axis 4
eigenvalues	0.977	0.021	0.001	0.001
Cumulative percentage (%) variance of species data	97.7	99.8	99.9	100
Sum of all unconstrained eigenvalues	1.000			

Table 2.6 Stump larval population PCA ordination summary for sites colonised from 13 January to 3 March 1999.

Axes	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.911	0.076	0.007	0.003
Cumulative percentage (%) variance of species data	91.1	98.7	99.1	99.7
Sum of all unconstrained eigenvalues	1.000			

Table 2.7 Stump larval population PCA ordination summary for sites colonised from 13 January to 3 March 1999.

Axes	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.974	0.021	0.004	0.000
Cumulative percentage (%) variance of species data	97.4	99.5	99.9	100.0
Sum of all unconstrained eigenvalues	1.000			

Figure 2.16 PCA site ordination of stump larval populations in sites colonised from 18 March to 11 May 1998 showing the relationship between sites (note each point represents an individual stump).

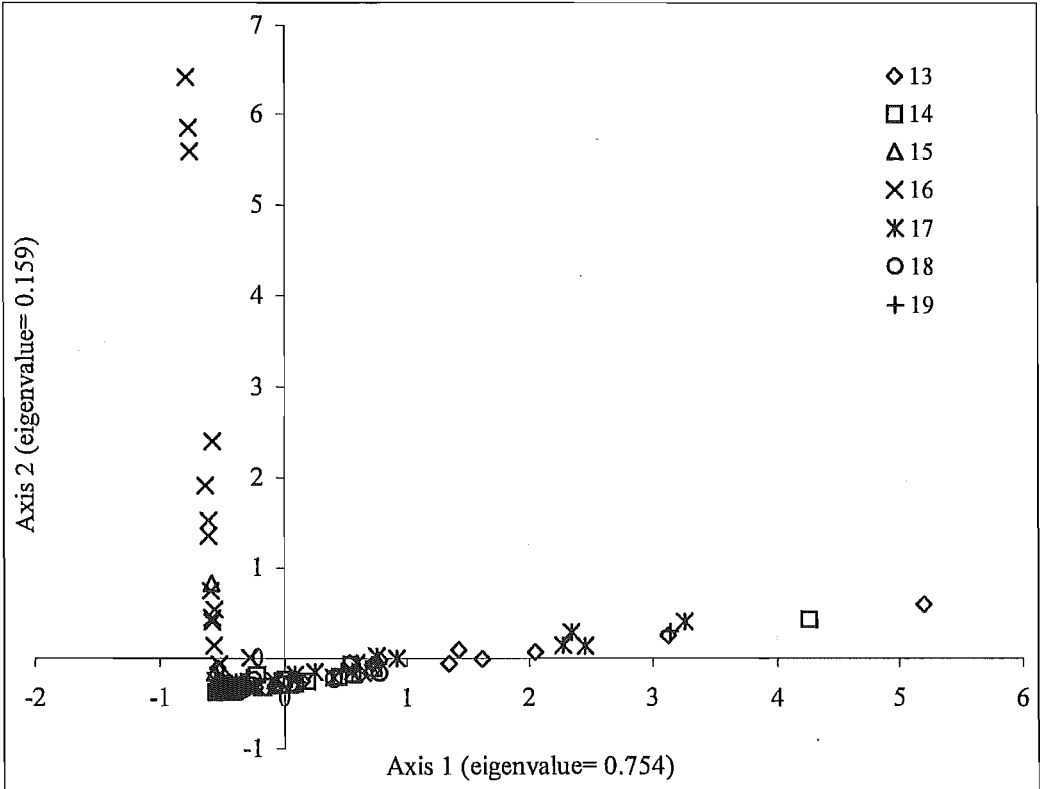


Figure 2.17 PCA species ordination of stump larval populations in sites colonised from 18 March to 11 May 1998 indicating the relative strength and direction of species effects.

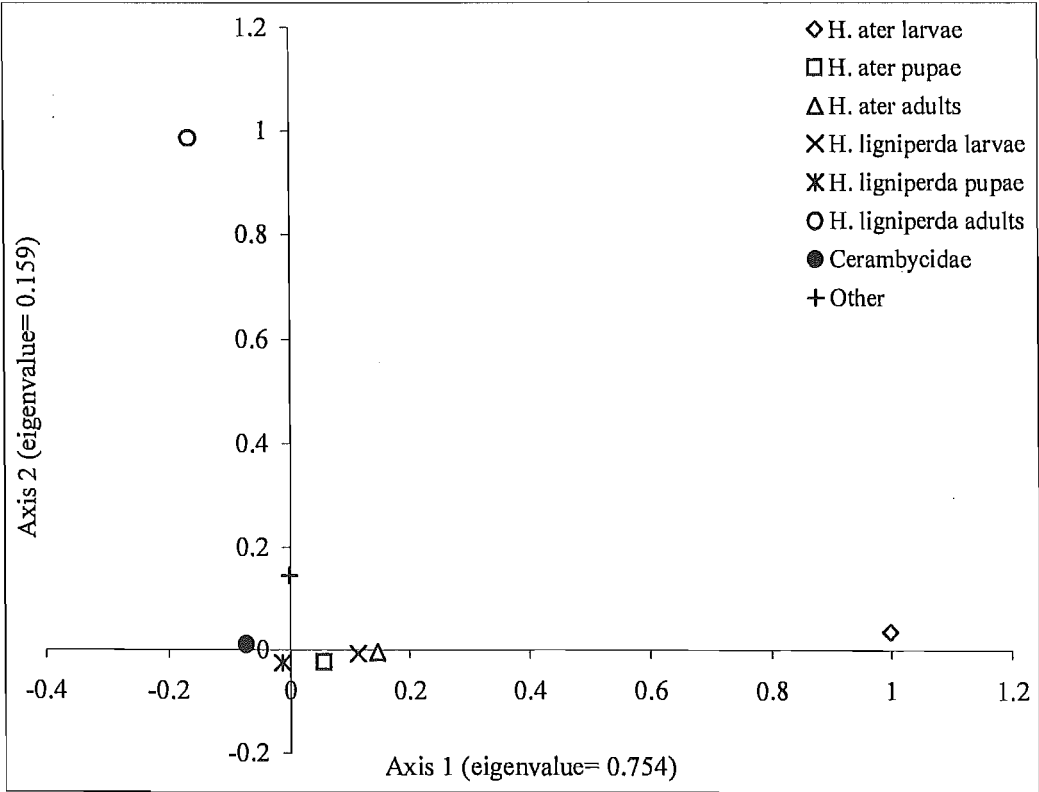


Figure 2.18 PCA site ordination of stump larval populations of sites colonised from 1 October 1998 to 25 November 1998 showing the relationship between sites (note each point represents an individual stump).

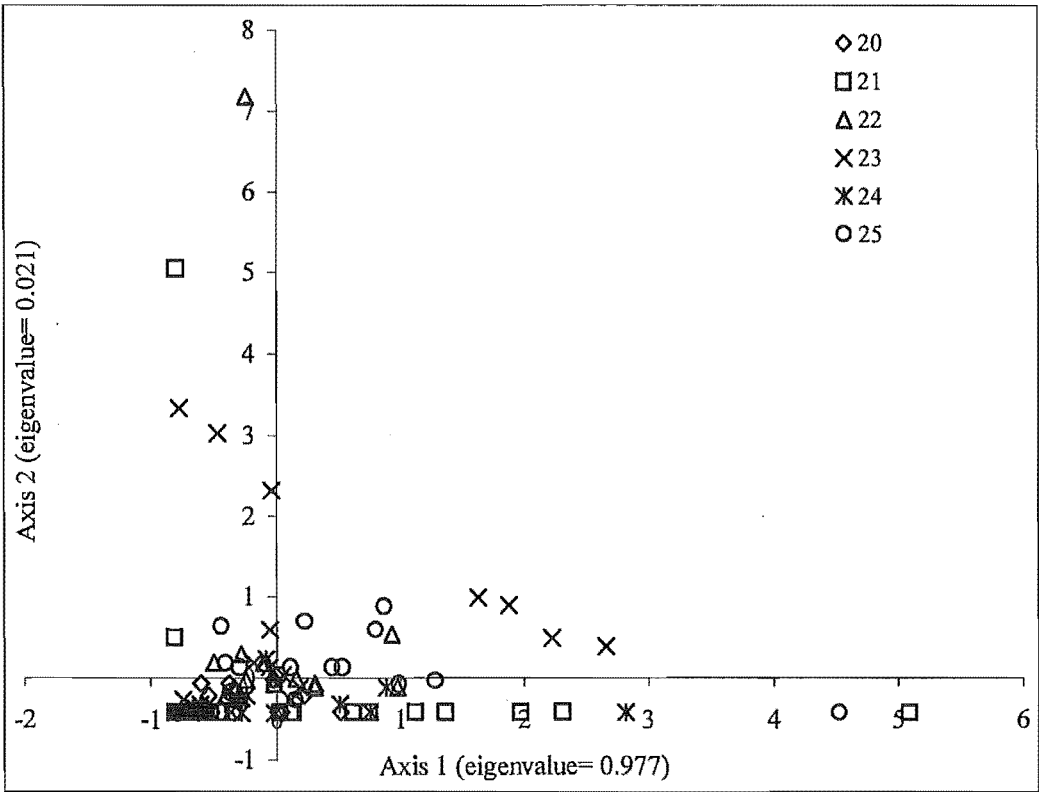


Figure 2.19 PCA species ordination of stump larval populations of sites colonised from 1 October 1998 to 25 November 1998 indicating the relative strength and direction of species effects.

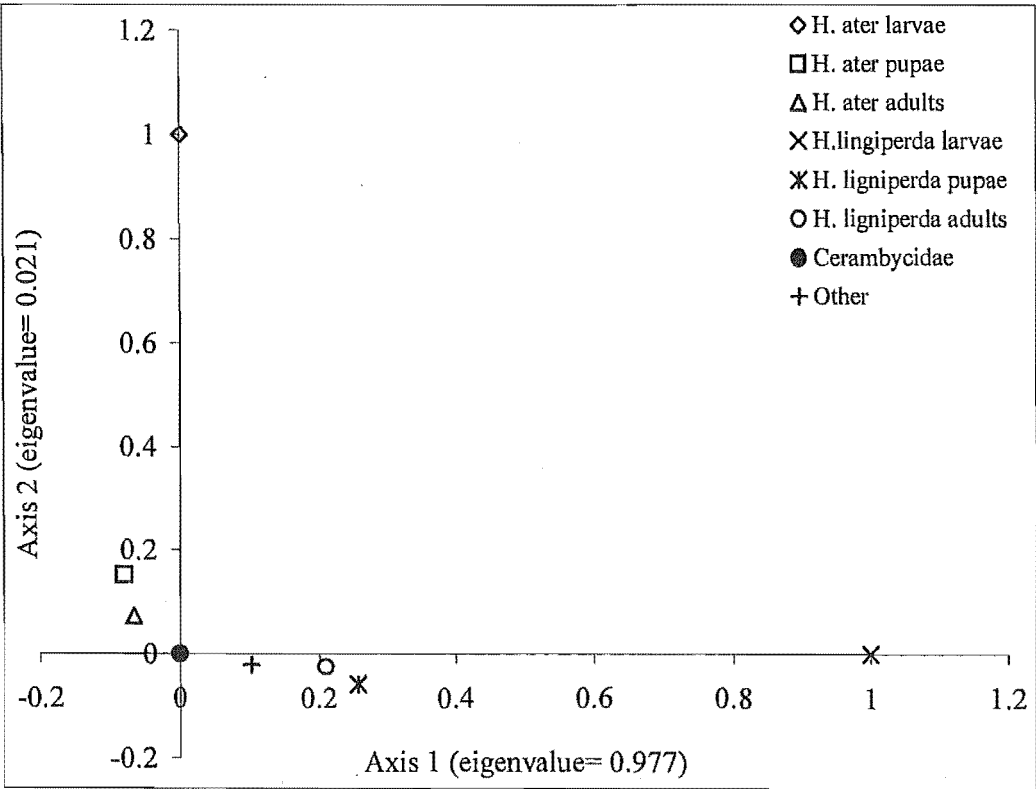


Figure 2.20 PCA site ordination of stump larval populations of sites colonised from 13 January to 3 March 1999 showing the relationship between sites (note each point represents an individual stump).

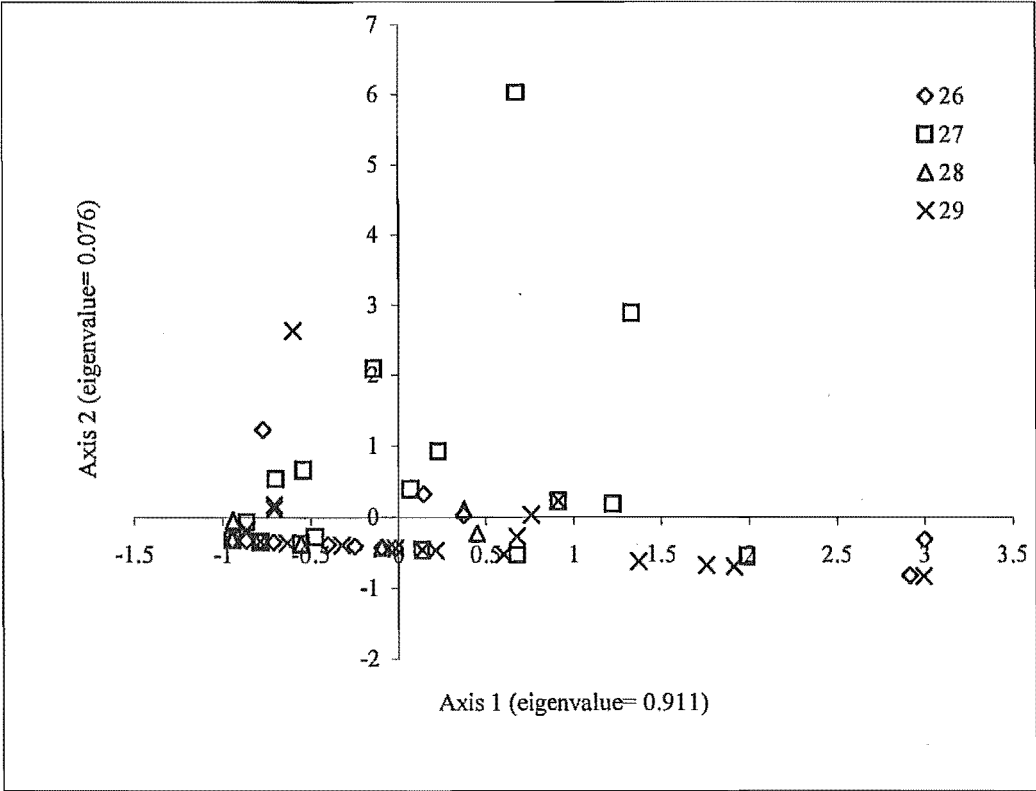


Figure 2.21 PCA species ordination of stump larval populations of sites colonised from 13 January to 3 March 1999 indicating the relative strength and direction of species effects.

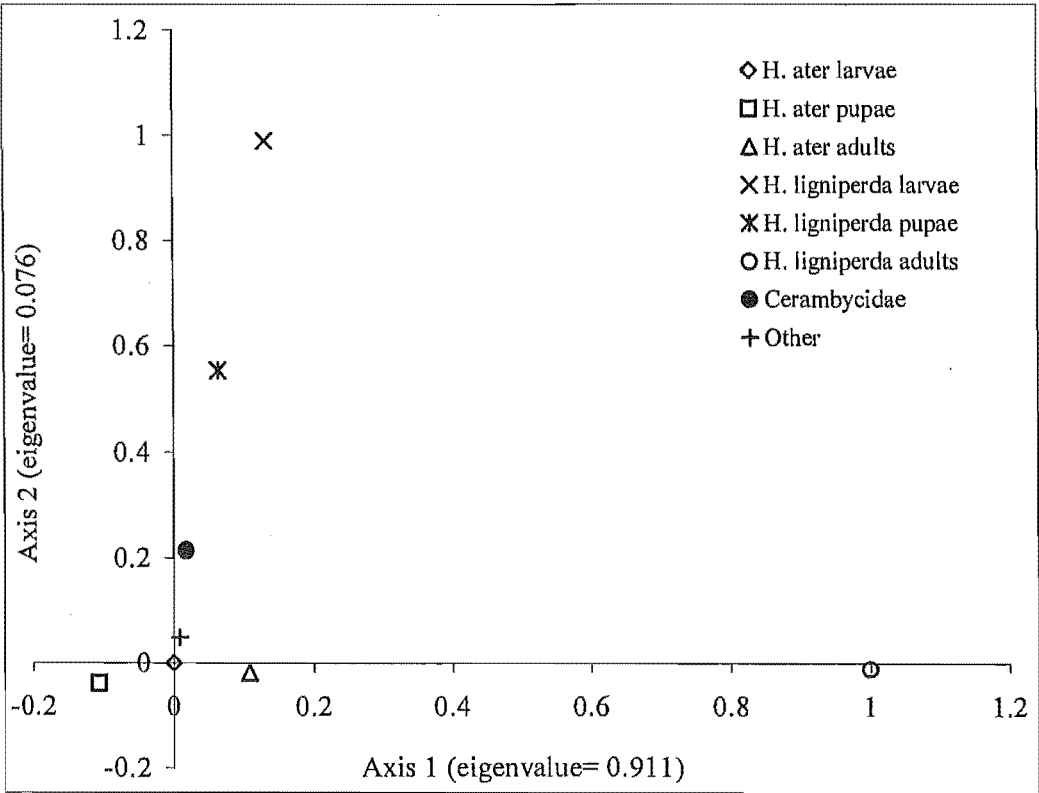


Figure 2.22 PCA site ordination of stump larval populations of sites colonised from 17 March to 5 May 1999 showing the relationship between sites (note each point represents an individual stump).

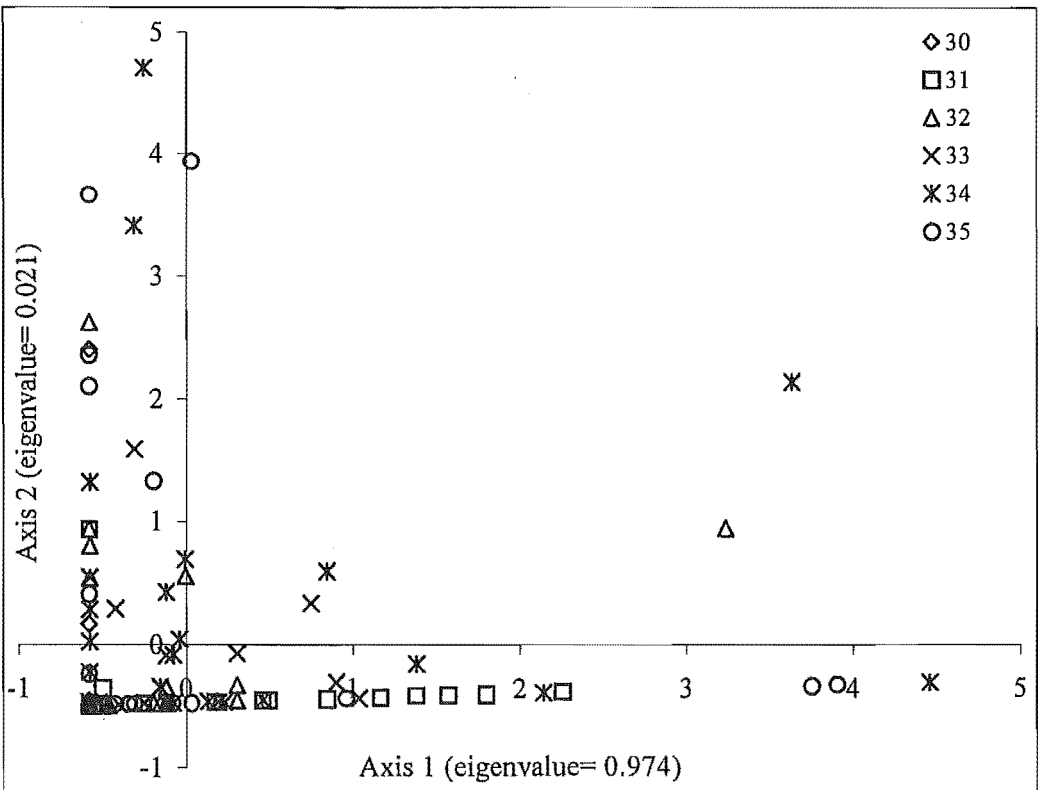
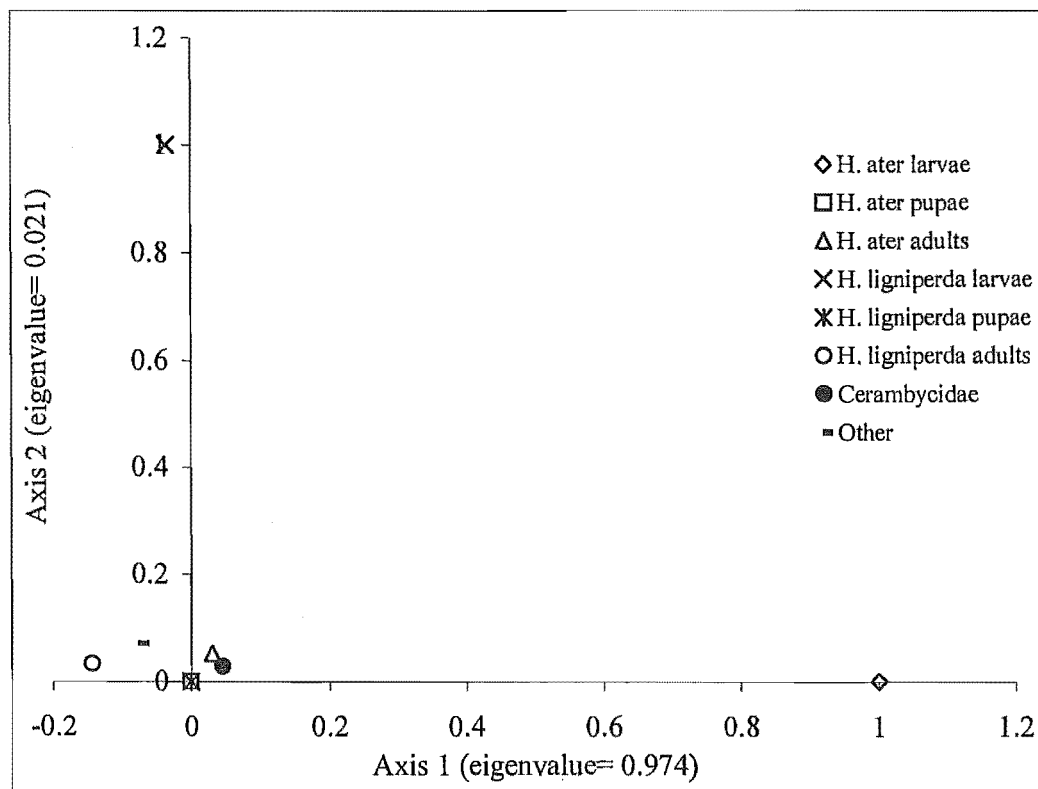


Figure 2.23 PCA species ordination of stump larval populations of sites colonised from 17 March to 5 May 1999 showing the relative strength and direction of species effects.



None of the variables measured had a strong effect on stump larval populations (Table 2.8). However, bark area had a weak positive effect on the first axis variation for the colonisation period 1 October to 25 November 1998. As a significant amount of variation is related to *H. ligniperda* larvae, this indicates that the amount of bark area may influence the number of larvae found in stumps.

Table 2.8 Spearmans rank correlation coefficients calculated between the stump and site environmental variables and the first two PCA stump larval population ordination axes for sites colonised during the colonisation four periods sampled.

	18 March to 11 May 1998		1 October to 25 November 1998		13 January to 3 March 1999		17 March to 5 May 1999	
Environmental variables	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
Mean no. <i>H. ater</i> colonising adults	0.022	0.094	0.053	0.168	0.239	-0.060	-0.032	0.244
Mean no. <i>H. ligniperda</i> colonising adults	-0.088	-0.233	-0.006	-0.065	0.225	-0.282	0.122	0.354
Stump height	-0.015	0.142	0.162	-0.165	0.363	-0.038	0.056	0.152
Stump diameter	-0.014	0.276	0.266	-0.054	0.020	0.044	-0.355	0.015
Outer bark cover	0.010	0.202	0.303	0.265	0.163	0.186	0.110	0.056
Bark area	0.007	0.326	0.423	0.068	0.252	0.167	-0.022	0.166
Inner bark condition	0.379	0.304	0.045	0.175	-0.061	0.192	-0.066	-0.305
Slash	-0.004	0.215	0.017	0.229	-0.179	0.079	0.237	0.031
Aspect	0.160	-0.010	0.044	0.354	0.097	0.115	0.236	0.215
Slope	-0.159	-0.146	0.004	0.233	0.097	0.115	0.091	0.181

H. ligniperda and *H. ater* were the dominant species in *P. radiata* stumps. Cerambycid species were the next most abundant group of insects sampled from stumps. Cerambycid larvae were found most often in sites harvested during the middle of the summer period. However, in comparison to *H. ater* and *H. ligniperda*, cerambycid numbers were low. Other species of insect larvae found in stumps included Buprestidae, Diptera and some predatory Coleoptera larvae. Members of these groups were rarely encountered.

The data indicate that the stump larval populations largely reflect the relative patterns of adult colonisation during the period following harvesting. While both species may be present in stumps at any time, the relative abundance of the larvae of each species

generally reflects the relative colonisation of each species. This indicates that like the adult populations, the larvae of *H. ater* and *H. ligniperda* were separated from each other in time. *H. ligniperda* larvae were the dominant species in the stumps of sites harvested during the winter period (when insignificant numbers of either species are active) through to the end of summer.

H. ater larvae dominated sites harvested over two months (during autumn). Harvesting activities create large areas of potential habitat for bark beetles continuously throughout the year in second rotation forests (Tribe 1990, 1991b, Wilson & Day 1995, Rieske & Raffa 1999). Breeding habitat created during the winter period was colonised by *H. ligniperda* during the peak period of spring emergence. Following this period of peak *H. ligniperda* activity there was a period (late November to January) where the activity of both species was low. Any habitat not colonised during this period by either species was colonised by the next peak of *H. ligniperda* activity, which lasted until mid April. *H. ater* larvae were found in significant numbers in areas harvested just prior to March through to end of April. There was only one peak of very high activity of *H. ater* adults in April. *H. ater* larvae were dominant in breeding material harvested just prior to this period. Larvae of *H. ater* and *H. ligniperda* were present in all sites all year round, but often at low levels. This indicates that there were populations of both species occupying the same habitat.

None of the site or stump environmental variables measured appeared to strongly influence larval populations. This may be a reflection of the lack of sensitivity in the data, and illustrates the difficulty of collecting large amounts of accurate ecological data when working with insects. The variation in stump populations within sites may be concealing the influence of site or stump variables on larval populations. A strong positive relationship between bark area and larval numbers might be expected with a larger sampling effort. The failure to identify a relationship between larval populations and environmental variables does not mean a relationship did not exist.

2.2.3 The emergence of *H. ater* and *H. ligniperda* from *P. radiata* stumps

2.2.3.1 Introduction

This study investigated the pattern of emergence of *H. ater* and *H. ligniperda* from stumps. These observations complement those made during the two previous studies, and contribute to a better understanding of the interactions between the two species.

The objectives of this study were to:

- Observe emergence of both species from breeding material
- Determine the duration of the emergence period
- Investigate periods of peak emergence activity
- Investigate whether emergence activity may be related to other factors
- Determine whether the relative emergence activity of both species in a site reflects the colonisation of a site and the subsequent larval populations

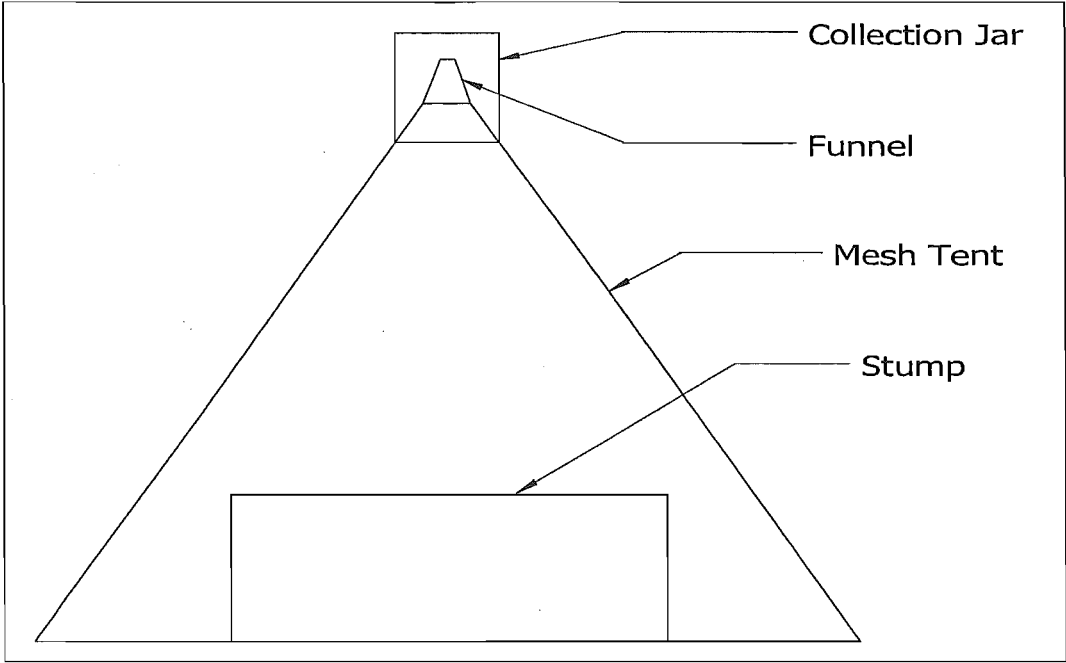
2.2.3.2 Methods

Two sites colonised from 1 October to 25 November 1998 (Sites 20 & 21) and two sites harvested prior to 17 March to 5 May 1999 (Sites 33 & 34) were selected (Appendix 1). Five stumps were selected in Sites 20 and 21. Six stumps were selected in Sites 33 and 34. The stumps were a similar size, but restricted by the size of the emergence traps. The maximum stump diameter was approximately 60 cm. Stumps were in good condition and had good bark cover. This was to ensure that stumps contained relatively high numbers of larvae and subsequently emerging beetles. Higher numbers of emerging beetles made patterns of emergence more detectable.

Emergence traps were made by constructing a cone shape nylon mesh ‘tent’ with a ‘jar’ on top to collect captured beetles (Figure 2.24). Very fine nylon mesh was used to ensure beetles were not able to escape. The large opening at the base of the tent was 70 cm in diameter. The upper opening was 10 cm in diameter. A collection jar was constructed by cutting a 1.5 litre plastic soft-drink bottle into three pieces. The top or neck of the bottle was inverted into the main part of the bottle, while the base of the bottle was fitted as a lid (Figure 2.24). The three parts of the collection jar were joined using heavy PVC tape. The

collection jar was taped to the mesh tent. The trap design ensured that beetles emerged from the stumps and made their way up the tent and into the collection jar. Beetles were unable to return to the tent once in the collection jar. The tent was attached to a 1.8m wooden stake.

Figure 2.24 Construction of emergence traps.



The emergence traps were serviced at the same time as the colonisation traps (refer to section 2.2.1.2). Servicing consisted of removing all captured insects. Collections were stored in 70% ethanol until sorting. The traps were a very cheap and effective way to collect beetles emerging from stumps. They were strong enough to withstand the elements during the trapping period. Despite being able to chew through bark, beetles did not attempt to chew through the nylon mesh.

2.2.3.3 Results and Discussion

H. ligniperda dominated the emergence activity from stumps sampled in Sites 20 and 21 (Figure 2.25). Emergence activity from stumps in these sites continued over three months. *H. ligniperda* began emerging 13 January 1999 and emergence continued until the trapping programme ended on 5 May 1999. *H. ater* emerged from stumps in these sites, but in low numbers. Emergence activity reflected the relative colonisation activity and the subsequent larval populations in these sites.

H. ligniperda emergence activity peaked for both sites during the middle of February 1999. The peak of emergence activity was one week earlier in Site 20 compared with Site 21 and indicates that there may be small differences in generation times or breeding cycles between sites. However, the period of peak activity in both sites was in synchrony with the period of peak *H. ligniperda* flight activity during the mid-summer period (refer to section 2.2.1.3).

The length of time for *H. ligniperda* (and most likely *H. ater*) adults to colonise stumps and for the resulting offspring to develop to maturity and emerge was three to four months during the first half of summer. While generation times may be faster during mid-summer when temperatures are warmer, it is probable that offspring produced by these emerging beetles would be unlikely to complete development and emerge before the winter period.

H. ater dominated the emergence activity from the stumps in Sites 33 and 34 colonised from 17 March to 5 May 1999 (Figures 2.26). The results indicate that emergence activity may continue over a reasonably long period of time. *H. ater* adults began emerging from stumps during November 1999. Emergence continued until the trapping programme ended on 20 April 1999. *H. ligniperda* emerged in similar numbers to *H. ater* in Site 33. In Site 34, *H. ater* dominated the emergence activity. Emergence activity in Sites 33 and 34 harvested prior to March 1999 largely reflected the relative colonisation activity and the subsequent larval patterns in these sites.

Considerable *H. ater* emergence activity continued in both sites for a period of three months, from 11 January 2000 prior to the peak of emergence activity during April 2000. This period of peak emergence was in synchrony with the period of peak *H. ater* flight

activity described above (refer to section 2.2.1.3). The peak emergence period for *H. ligniperda* was during the later part of February 2000. The peak of emergence activity was considerably smaller in Site 34.

The length of time for *H. ater* and *H. ligniperda* to colonise sites and for their offspring to complete development to maturity was 10-13 months for sites colonised at the end of summer. The offspring of colonising adults overwintered as larvae. Development of the overwintering larvae continued during the summer period. The summer development of overwintering larvae was slower compared with larvae resulting from spring oviposition. This and the long period of emergence activity for adults of both species in these sites indicate that adults may remain inactive beneath the bark for a considerable period of time before emerging. Emergence activity may be triggered by some environmental cue, resulting in synchronised emergence.

Figure 2.25 Mean weekly emergence activity of *H. ligniperda* and *H. ater* adults from five stumps in Sites 20 and 21 colonised from 1 October to 25 November 1998.

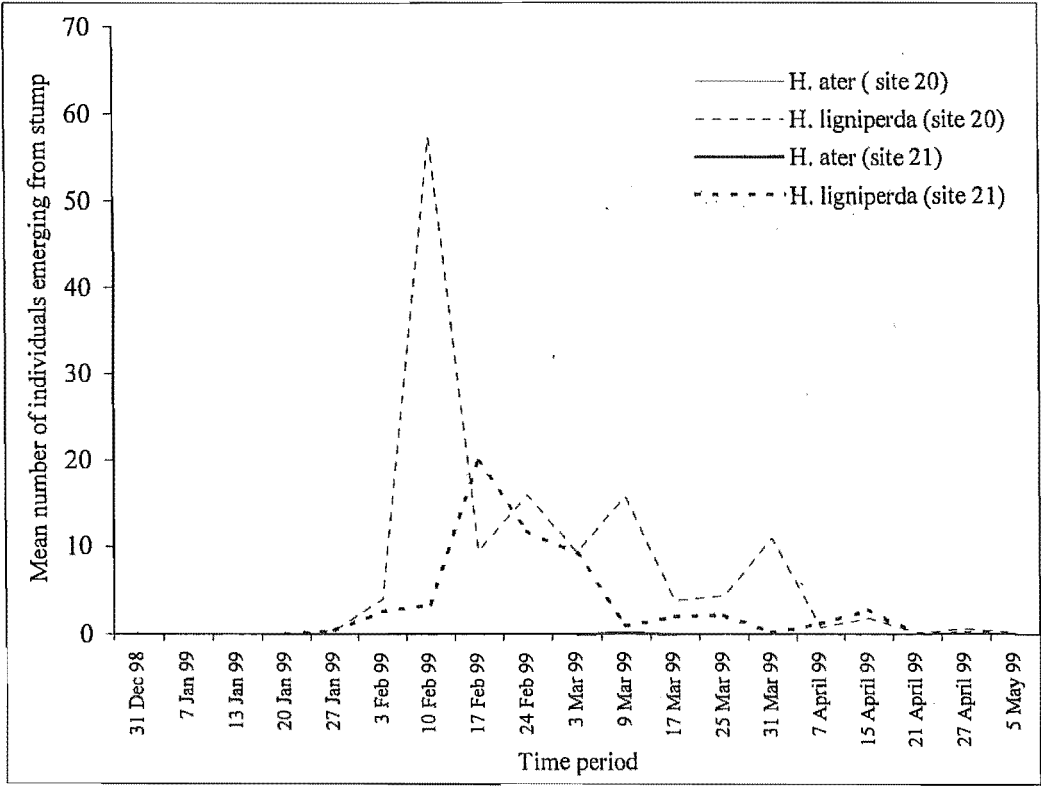
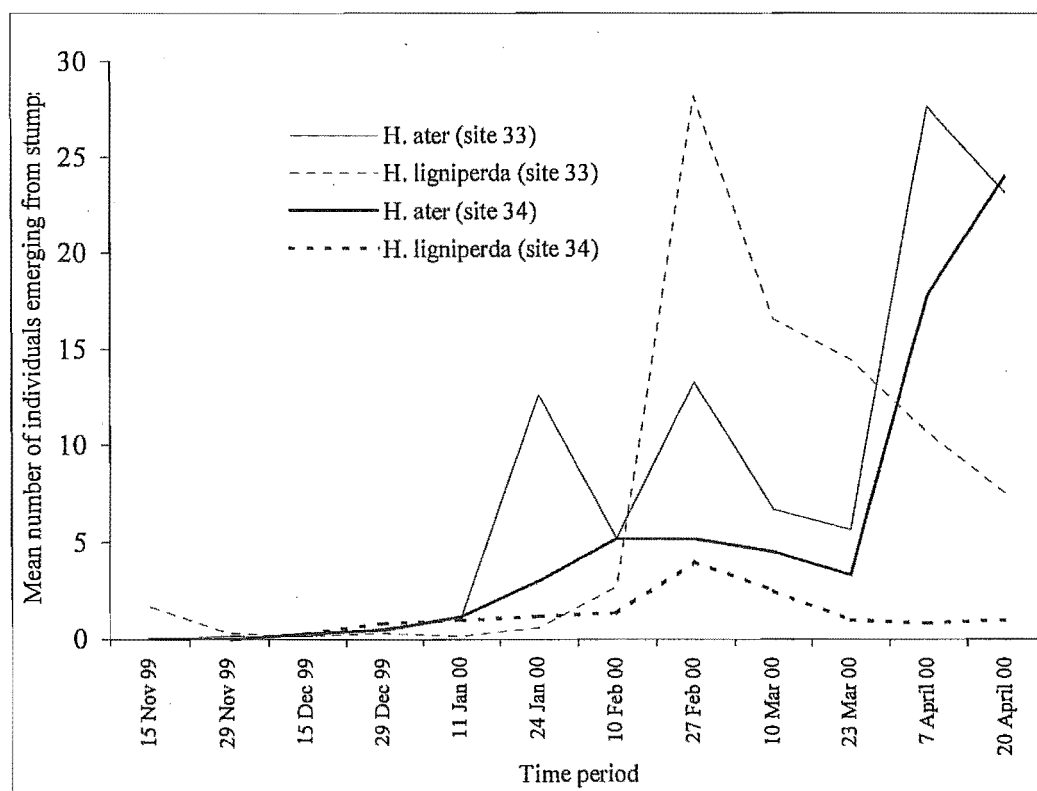


Figure 2.26 Mean fortnightly emergence activity of *H. ligniperda* and *H. ater* adults from six stumps in Sites 33 and 34 colonised from 17 March to 5 May 1999.



2.3 Conclusion

Both species co-existed in the same breeding material, yet also had discrete and identifiable periods of peak activity. These periods of peak activity and the subsequent offspring produced by colonising adults for each species were separated from each other in time. During one species period of dominance, the other species was present in low numbers. A similar situation occurs in South Africa, with *Hylastes angustatus* and *Hylurgus ligniperda* (Tribe 1990, 1991a,b). In South Africa the activity of *H. angustatus* is characterised by one sharp peak of activity per year, following a period of spring emergence in September and October (Tribe 1990). This period of activity accounted for 95% of the annual catch of *H. angustatus*. In South Africa, *H. ligniperda* has peak of activity during April and May, but this accounts for only 37% of the annual catch (Tribe 1991a). The flight activity of this species is variable throughout the year (Tribe 1991b). A third species of pine bark beetle is present in South Africa, *Orthotomicus erosus* (Woll.) and its activity peaks vary from year to year, but are always between October and February (Tribe 1991a).

Observations regarding *H. ater* in New Zealand were similar to those of *H. angustatus* in South Africa made by Tribe (1991b). However, the activity of *H. angustatus* peaks in spring (Tribe 1990) whereas the peak activity of *H. ater* was in autumn. The peak period of *H. ligniperda* in South Africa is during autumn (when the activity of *H. ater* in New Zealand was highest) (Tribe 1991a). In New Zealand *H. ligniperda* was most active in spring, although there were other periods of activity during the summer period. In New Zealand the annual variability of *H. ligniperda* was consistent with that observed in South Africa (Tribe 1991b). In France, the major peak of *H. ligniperda* activity is in spring with a smaller peak in autumn (Fabre & Carle 1975), which is more representative of the New Zealand situation. The activity peaks of each species in a different season indicates that different species may be responding to different environmental cues (Tribe 1991b).

The observations made during these studies indicate that the life cycle and development times for *H. ater* are similar to those suggested by Crowhurst (1969). Clark (1932b) and Crowhurst (1969) indicated that there were three overlapping broods of *H. ater* per year in New Zealand. However, the establishment of *H. ligniperda* in New Zealand has resulted in the dominance of this species during the summer months. Ciesla (1988) reported that *H. ligniperda* is dominant over *H. ater* in Chile. Observations by Tribe (1990, 1991a,b) indicate that *H. ligniperda* is the most dominant species in South Africa. One effect of the introduction of *H. ligniperda* has been to mask the activity of *H. ater* during the summer period. Population dynamics of *H. ater* were difficult to observe when the species was present at low levels.

Crowhurst (1969) reports that overwintering *H. ater* larvae enter a true diapause, which is broken in early January. Larvae from eggs laid by *H. ater* during early spring were also observed to pupate in January. Only larvae derived from eggs laid in March and April enter this period of diapause (Crowhurst 1969). The larval resting stage prior to pupation is up to two weeks for larvae from eggs oviposited at all other times of the year (Crowhurst 1969). Crowhurst (1969) suggests that the function of this larval diapause means that overwintering larvae and those larvae that develop during the summer reach maturity at the same time, during January. This means that adults from 'fast' and 'slow' broods are not segregated from each other. Crowhurst (1969) suggests that if segregation were to occur it might lead to the separation of *H. ater* into two species. However, neither of the 'new' species resulting from this segregation would have the capacity of the existing *H. ater*

species to adapt to a range of climatic conditions (Crowhurst 1969). In addition to fast and slow developing larvae maintaining synchrony, these developing larvae reach maturity during the warmest period of the year. This means that the larval offspring produced by these adults will develop during the period when conditions are optimal (Crowhurst 1969). These observations by Crowhurst (1969) indicate peak flight activity occurred during January. Neither Clark (1932b) nor Crowhurst (1969) report the relative level of *H. ater* activity during the year.

The results confirm observations made by Crowhurst (1969) regarding diapause in overwintering larvae. However, the period of peak emergence activity during January, as implied by Crowhurst (1969), did not occur until April. This study showed that while some overwintering larvae may develop and emerge during January, this was not normal. Overwintering larvae did not begin to emerge in great numbers until March. This means the main period of *H. ater* emergence was approximately three months later than the period suggested by Crowhurst (1969). The observations made during this study indicate that newly developed adults remain in stumps for longer periods prior to emergence than suggested by Crowhurst (1969).

Following Crowhurst's (1969) observations on *H. ater*, *H. ligniperda* was introduced into New Zealand. As *H. ligniperda* flight activity peaks during the mid-summer period, it is possible that the delayed emergence by *H. ater* is in response to competition from *H. ligniperda*. While both species are able to occupy the same habitat, *H. ligniperda* appears to be more competitive. A competitive advantage by *H. ligniperda* over *H. ater* is most apparent during the summer months when populations of *H. ligniperda* are clearly dominant. Presumably *H. ater* would have colonised all habitat created during the summer months before *H. ligniperda* was introduced into New Zealand in 1974. Breeding habitat resulting from harvesting during the summer is now dominated by *H. ligniperda*.

While interactions between *H. ater* and *H. ligniperda* were investigated in these studies, they could not be studied in detail. An objective of this programme was to investigate the relationship between the two species and to determine whether any interaction may have an influence on the potential pest status of *H. ater* in New Zealand. Further work is required to investigate interactions between *H. ater* and *H. ligniperda* in order to

understand factors that influence populations and drive competition between the two species.

In the natural situation for both *H. ater* and *H. ligniperda*, access to breeding material is likely to be limited during endemic conditions (i.e. an occasional tree fall) (Raffa & Berryman 1983, Tribe 1991b, Kirkendall *et al* 1997). Secondary or non-aggressive bark beetles are very successful at exploiting such habitat (Tribe 1991b, Wilson & Day 1995, Örlander *et al* 1997, Rieske & Raffa 1999). In endemic situations large-scale disturbances would result in an increase of breeding habitat. These bark beetles are able to quickly exploit an increase in available substrate and populations will reach epidemic proportions, until the increased supply of breeding material is exhausted (Rudinsky 1962, Berryman 1972, Christiansen *et al* 1987). In New Zealand *P. radiata* forests large-scale harvesting activities are continual year round. A consequence of this is that enough breeding habitat is being created for populations levels to reach epidemic proportions, and be sustained at these levels (Leather *et al* 1999).

Observations made during this study indicate that the vast majority of stumps created during harvesting activities were colonised by either *H. ater* or *H. ligniperda*, or both species. The continuous creation of new habitat means that new generations of beetles are also being continuously initiated. Consequently, all life stages of the insects are present all year round. However, during some periods certain life stages may be more abundant than others as illustrated during periods of peak flight activity. Historically, all breeding material would have been occupied by *H. ater* in New Zealand. *H. ligniperda* has not been observed to attack *P. radiata* seedlings in New Zealand (Bain 1977a, personal observations). Therefore the introduction of *H. ligniperda* has probably had some influence on the pest status of *H. ater*. *H. ligniperda* is the dominant species and occupies the majority of habitat created during harvesting activities. Consequently, the activity of *H. ater* has decreased. It is reasonable to assume that the impacts of *H. ater* on the forest industry will also have reduced.

Unfortunately, little detailed documentation is available with respect to the historical impacts of *H. ater* in New Zealand. As forestry practices have changed dramatically over recent years, the perceived pest status of *H. ater* has also changed. Changes in forestry

techniques and practices have probably impacted on the biology of *H. ater*. It was an ideal time to reassess the threat that *H. ater* posed to the forest industry in New Zealand.

This Chapter indicates that changes in the biology and ecology of *H. ater* since the introduction of *H. ligniperda* were likely. Therefore it seems appropriate to investigate whether such changes may have impacted on the amount of damage caused by *H. ater*.

The remainder of this thesis investigates the effects that these changes in ecology and behaviour of *H. ater* (following the introduction of *H. ligniperda*) have had on its pest status. It was possible to quantify the extent of the problem due to *H. ater* by determining the amount of attack on seedlings by *H. ater*, and the implications of this attack to the *P. radiata* forest industry. Once the impacts of *H. ater* were investigated then strategies could be developed to control *H. ater*, or to manage the risks associated with it.

3. THE PRIMARY EFFECT OF *H. ater* ATTACK ON SEEDLINGS

3.1 The incidence of *H. ater* attack and mortality in second rotation *P. radiata* forests in the central North Island, New Zealand

3.1.1 Introduction

In New Zealand large areas of mature *P. radiata* forest are harvested all year round. The stumps that result from these harvesting activities create a supply of breeding habitat that would not normally exist in the natural forest environment (Örlander *et al* 1997, Leather *et al* 1999). This means that *H. ater* populations are able to persist at epidemic levels for longer periods of time than would be expected in the natural forest environment. Adults emerge from stumps following larval development and begin maturation feeding on any available food material. It is during this maturation feeding that *H. ater* attacks seedlings planted following harvesting operations.

Seedling death resulting from bark beetle attack has not been well documented in New Zealand and was reviewed in Chapter One. Seedling mortality resulting from attack by other species of bark beetles and weevils is reasonably well documented (Du Toit 1975, Tribe 1990, Eidmann 1992, Wilson *et al* 1996, Leather *et al* 1999, Örlander & Nilsson 1999, Rieske & Raffa 1999). Lindelöw (1992) reported patchy mortality of Norway spruce (*Picea abies* L.) seedlings resulting from *Hylastes cunicularius* and *Hylobius abietis* attack in Sweden. Lindelöw (1992) observed that mortality was often undetected as dead seedlings were seldom found, and when they were, the causes of death were often misdiagnosed. Seedling mortality in excess of 30% was common (Lindelöw 1992). *Hylastes angustatus* was reported to kill *Pinus patula* seedlings in South Africa (Atkinson & Govender 1997). While occasional high levels of mortality and frequently lower levels of mortality were reported, there has been little quantitative assessment (Atkinson & Govender 1997). Corrective treatment of seedling attack by bark beetles is not possible once damage is apparent (Atkinson & Govender 1997). In Britain, *Hylobius abietis* is

reported to kill 30-100 % of all *Picea* seedlings planted in restocking sites and is therefore a serious threat to British and European forestry (Leather *et al* 1999).

Lindelöw (1992) and Leather *et al* (1999) discuss attempts to identify characteristics of sites that may make seedlings more susceptible to mortality by *Hylobius abietis* and *Hylastes cunicularis*. A wide range of site characteristics has been investigated with relatively little success (Lindelöw 1992, Leather *et al* 1999). If a relationship can be established between high levels of attack by *H. ater* and particular site characteristics, then high-risk sites may be treated prior to planting. Treatment may be management or control-based, depending on the costs and benefits associated with each, and the potential costs of the expected *H. ater* damage.

Seedling mortality is regarded as being the main problem associated with *H. ater* in New Zealand (Zondag 1958, 1968, Bain 1973, 1978). Seedling mortality is usually attributed to factors such as drought or poor planting and *H. ater* is reported to only select weak or stressed seedlings (Zondag 1958, 1968, Bain 1973, 1978). *H. ater* does not build up high populations in all areas (personal observations). Given the uncertainty surrounding the pest status of *H. ater* in New Zealand it was important to determine the amount of mortality in first year plantings that could be attributed to *H. ater* attack. This would clarify whether *H. ater* was a dominant cause of mortality in the early establishment of plantings, or whether other factors (environmental and/or biological) were responsible.

The objectives of this study were to:

- Determine levels of seedling mortality directly attributable to *H. ater* attack in second rotation *P. radiata* forests
- Determine whether seedling mortality due to *H. ater* attack was the dominant factor causing seedling mortality in the first year of establishment
- Determine the frequency and severity of sub-lethal attack on one year old seedlings by *H. ater* in second rotation forests
- Examine the relationship between the attack of seedlings by *H. ater* and any subsequent mortality, and investigate what factors (biological and/or environmental) may contribute
- Identify sites where seedlings were at high risk of *H. ater* attack

3.1.2 Methods

Second rotation *P. radiata* sites that had been planted during the winters of 1998 and 1999 were selected in Kinleith and Kaingaroa forests. Sites were chosen to include a variety of harvesting histories and site types. Information on harvesting activities and site factors were provided by Carter Holt Harvey Forests and Fletcher Challenge Forests. Sampling was undertaken during late autumn, approximately nine months following planting.

Sites were sampled by randomly locating a transect in each site. Depending on the spacing of seedlings in each site, the length of each transect was between 300-600m. Differences in length were due to differences in initial stocking rates between sites. One hundred seedlings encountered along the transect were recorded as being either dead or alive. All dead seedlings encountered were removed from the soil and examined for evidence of *H. ater* attack. Mortality was only attributed to *H. ater* if symptoms of attack were considered to be severe (as described below).

H. ater attacks the roots and collars of seedlings below the ground (Clark 1932b, Crowhurst 1969, Zondag 1958). In almost all cases, it was not possible to observe wounding as a result of *H. ater* attack without removing the seedling from the ground. Every fifth seedling encountered was destructively sampled regardless of condition. In total, twenty seedlings were destructively sampled in each site. Each of these was examined for evidence of *H. ater* attack. ‘Attack’ was evidence of feeding activity by *H. ater*. ‘Severity of attack’ was the extent to which *H. ater* attacked a seedling. The severity of attack on seedlings was recorded as follows:

0= No evidence of *H. ater* attack (Photo 3.1)

No evidence of feeding activity was observed on the root collar and the roots of seedlings.

1= Mild *H. ater* attack (Photo 3.2)

Small spots of resin around the root collar or on roots indicated an attempt by *H. ater* to feed. In some cases, feeding attempts were not severe enough to initiate a resin response by the seedling. Small “dots” of chewing activity indicated these feeding attempts.

2= Moderate attack by *H. ater* (Photo 3.3 and 3.4)

Moderate attack was recorded when either many feeding attempts (described above) were observed, or when there was evidence of one or two more sustained feeding attempts. For example, when feeding activity resulted in the removal of an area of bark greater than 1cm^2 .

3= Severe attack by *H. ater* (Photo 3.5 and 3.6)

Severe attack was recorded if ring barking or multiple feeding attempts covering the root collar and stem of the seedling by *H. ater* were observed.

Differences with respect to seedling mortality and the frequency of attack between sites were investigated using chi-square tests, using the statistical package SAS (PROC FREQ, Version 6.12 for Windows, SAS Institute 1996). Differences with respect to the severity of attack between sites were investigated using analyses of variance (ANOVA), using the statistical package SAS (PROC GLM, Version 6.12 for Windows, SAS Institute 1996). Pairwise multiple comparisons were conducted using Duncan's multiple range tests to determine the nature of the differences detected by ANOVA.

Photo 3.1 *P. radiata* seedling showing no evidence of *H. ater* attack as described above.



Photo 3.2 *P. radiata* seedling showing mild *H. ater* attack as described above.



Photo 3.3 *P. radiata* seedling showing evidence of moderate attack by *H. ater* as described above.



Photo 3.4 *P. radiata* seedling showing evidence of moderate attack by *H. ater* as described above.



Photo 3.5 *P. radiata* seedling showing evidence of severe attack by *H. ater* as described above.

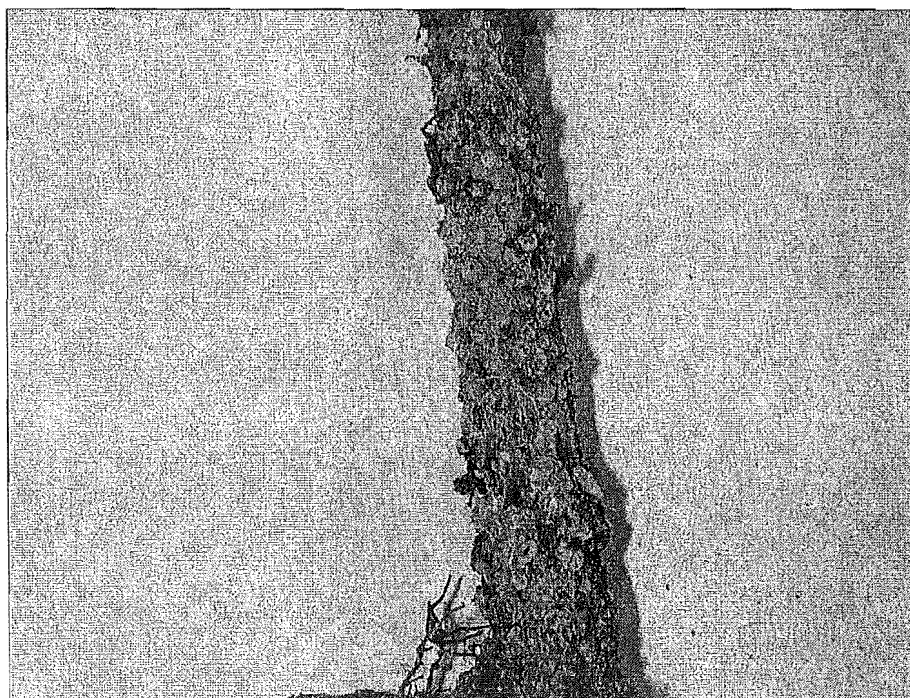
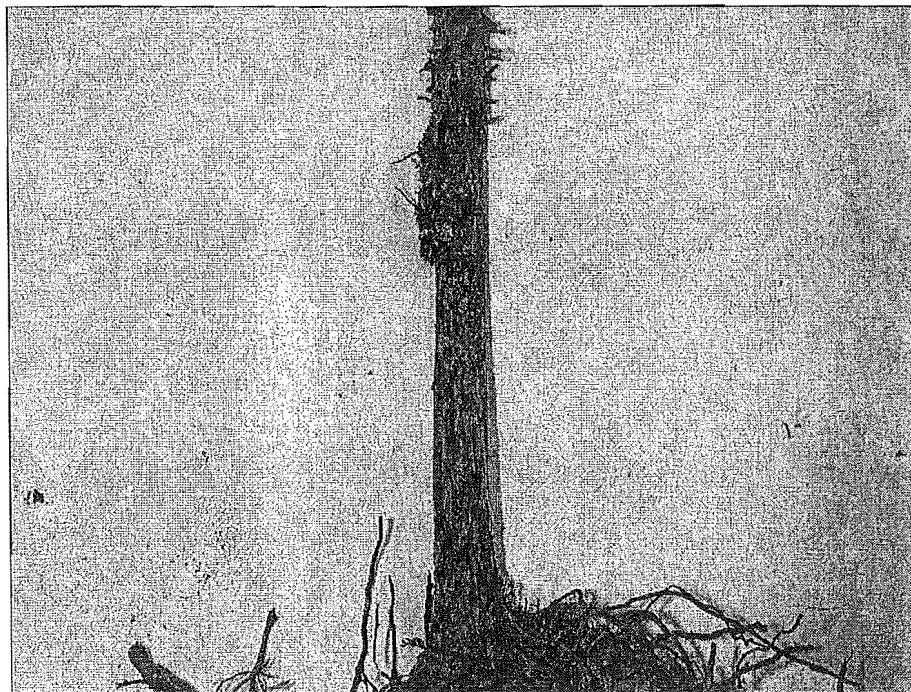


Photo 3.6 *P. radiata* seedling showing evidence of severe attack by *H. ater* as described above.



3.1.3 Results

Mortality resulting from *H. ater* attack

Thirty-two sites planted during the winter of 1998 were sampled. The number of seedlings killed by *H. ater* ranged from 0-30% and was significantly different between sites ($X^2_{(31)} = 300.762$, $P < 0.001$, Figure 3.1). Mean seedling mortality due to *H. ater* attack was 4.47% across the 1998 plantings. Mean seedling mortality due to unknown causes and those not attributable to *H. ater* attack was 1.97% and was significantly different between sites ($X^2_{(31)} = 205.684$, $P < 0.001$). Mortality due *H. ater* attack was significantly greater than mortality due to other causes ($X^2_{(1)} = 32.101$, $P < 0.001$). While most sites planted in 1998 were not effected by mortality resulting from *H. ater* attack, or low levels of *H. ater* related mortality (less than 5%), a small number of sites were more seriously effected (Figure 3.1).

Twenty-eight sites planted during the winter of 1999 were sampled. The amount of seedling mortality due to *H. ater* attack ranged from 0-13% and was significantly different between sites ($X^2_{(27)} = 150.179$, $P < 0.001$). Mean mortality attributable to *H. ater* in these plantings was 2.46%, considerably less than the previous year. Mean mortality not attributable to *H. ater* attack was 0.64%, also less than the previous year and differed between sites ($X^2_{(27)} = 53.902$, $P < 0.001$). Mortality due to *H. ater* was significantly greater than seedlings which died from other causes ($X^2_{(1)} = 30.368$, $P < 0.001$). As with the 1998 plantings most sites were not greatly effected by *H. ater* seedling mortality (less than 5%). Only a few sites were more seriously effected (Figure 3.2).

Figure 3.1 Amount of seedling mortality due to *H. ater* attack for 32 sites planted in the winter of 1998.

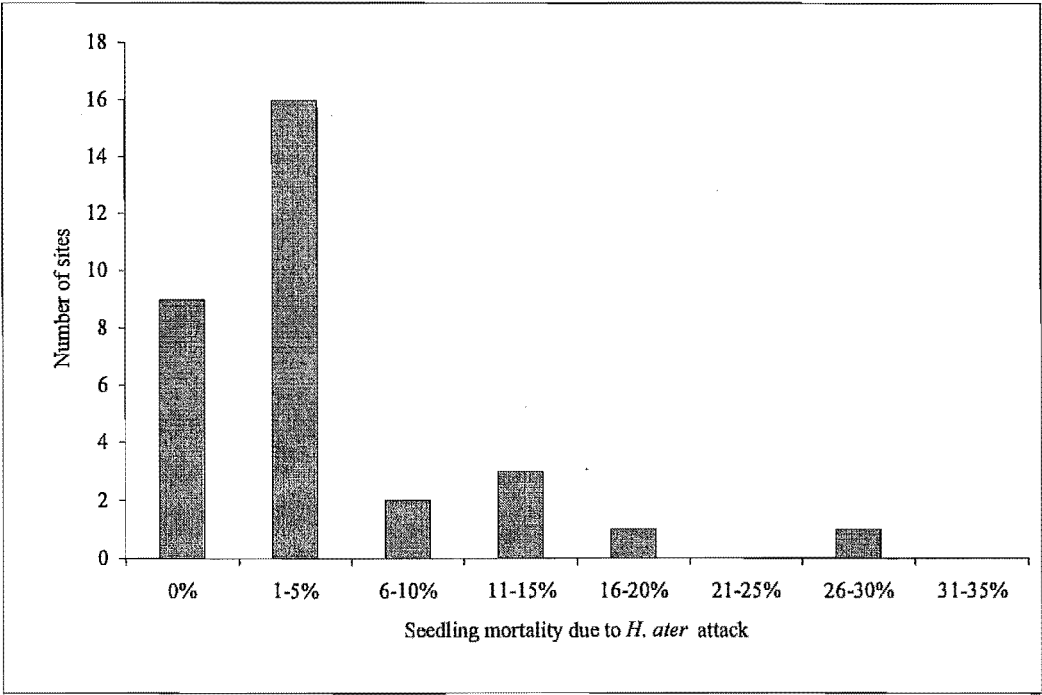
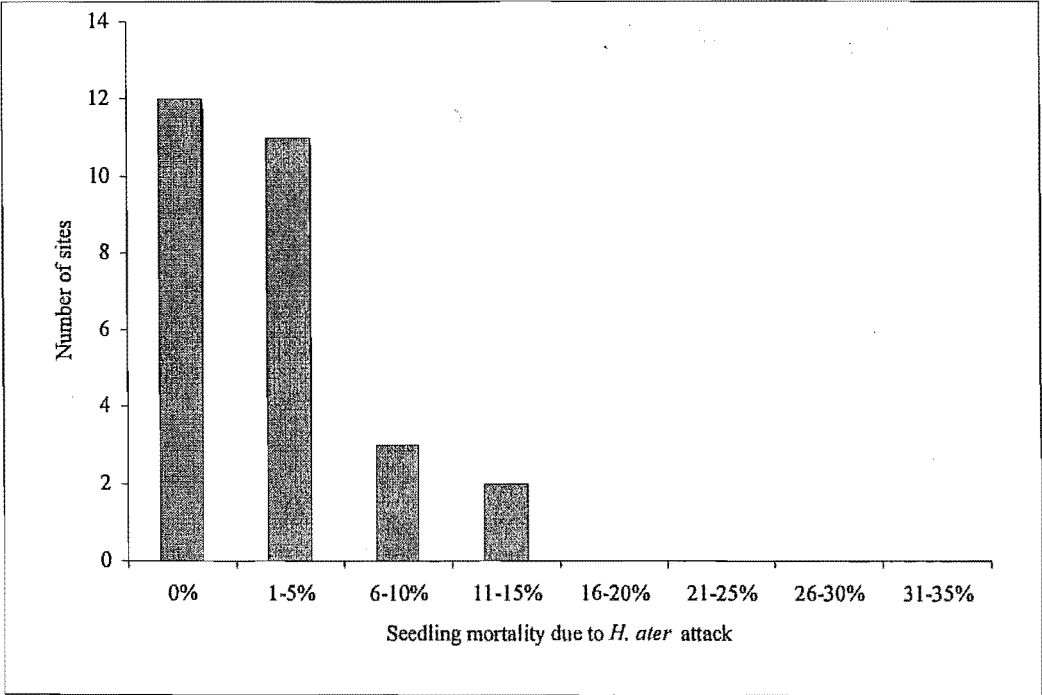


Figure 3.2 Amount of seedling mortality due to *H. ater* attack for 28 sites planted during the winter of 1999.



The frequency of seedling attack by *H. ater*

Attacks on seedlings by *H. ater* ranged from 0-90% for the 1998 plantings (Figure 3.3), and varied between sites ($\chi^2_{(31)} = 222.466, P < 0.001$). Seedlings planted during the winter of 1999 were attacked by *H. ater* with frequencies from 0-75% (Figure 3.4). Frequency of attack differed significantly between sites ($\chi^2_{(27)} = 116.568, P < 0.001$). While seedling mortality due to *H. ater* attack was usually low, there was a substantial amount of feeding activity in most sites. In 28% of sites planted in 1998, the frequency of attack was greater than 50% (Figure 3.3). In 21% of sites planted in 1999, the frequency of attack was greater than 50% (Figure 3.4).

Figure 3.3 Frequency of seedling attack by *H. ater* for 32 sites planted during the winter of 1998.

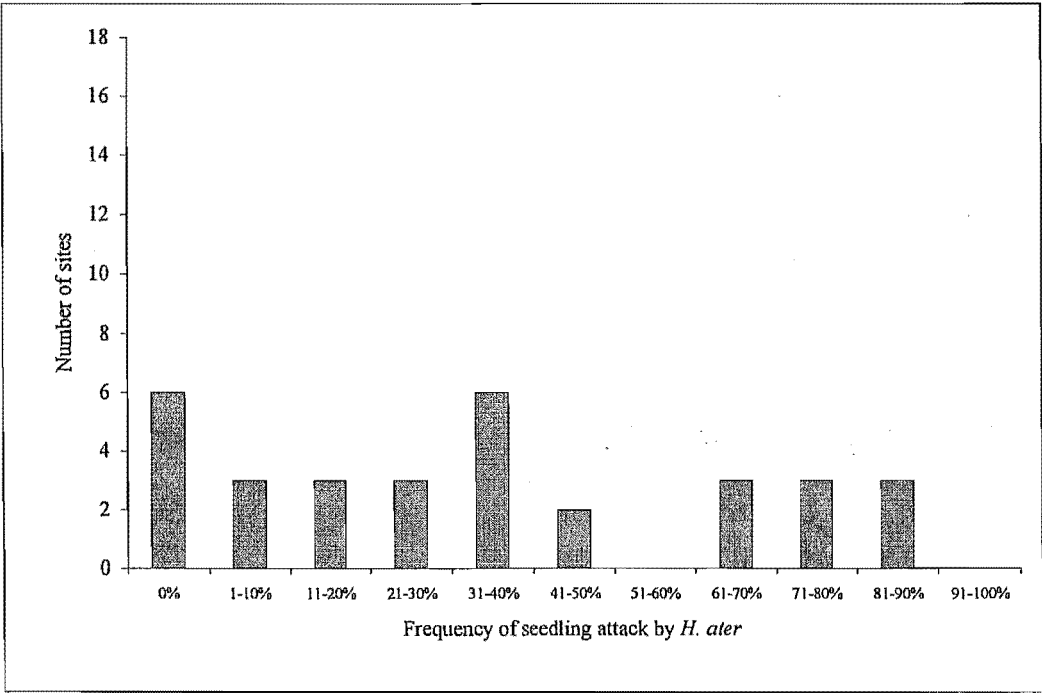
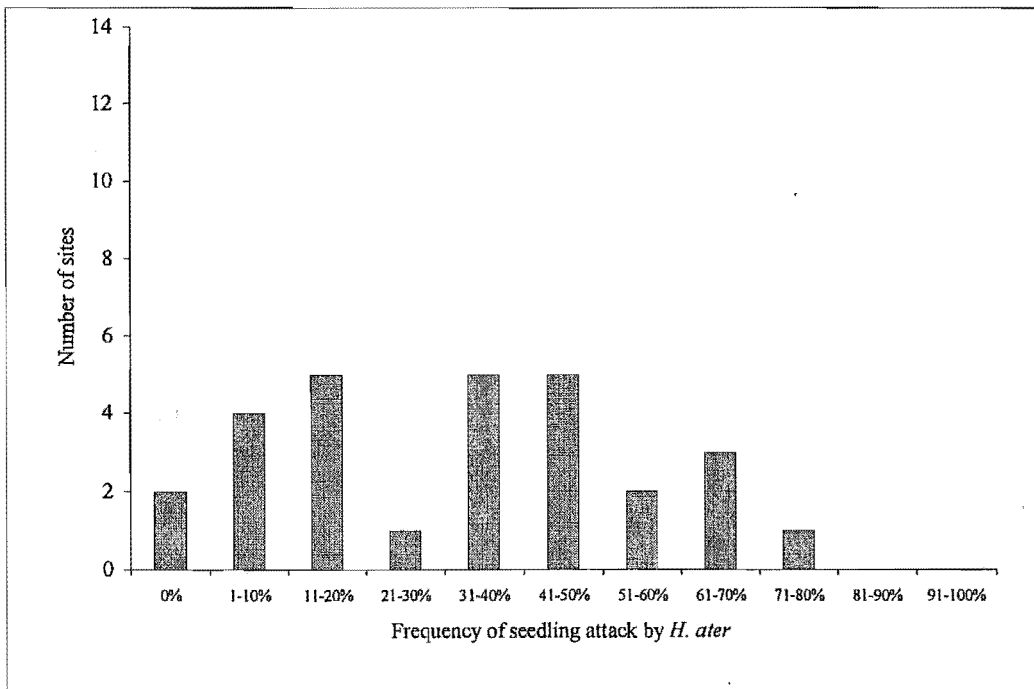


Figure 3.4 Frequency of seedling attack by *H. ater* for 28 sites planted during the winter of 1999.



The severity of seedling attack by *H. ater*

Mean severity of attack by *H. ater* differed between sites planted during 1998 ($F_{(31,607)} = 11.35$, $P < 0.001$, Figure 3.5). Mean severity of attack ranged from 0-2.2. Mean severity of attack of 2.2 indicates that the average seedling attack by *H. ater* was greater than moderate (refer to section 3.1.2). For the site with the greatest *H. ater* attack, 55% of the seedlings sampled showed evidence of severe attack. Five percent of seedlings and 25% of seedlings were attacked with mild and moderate severity, respectively. The remaining 15% of seedlings in this site were not attacked by *H. ater*.

Mean severity of attack by *H. ater* was different between sites planted in 1999 ($F_{(27,532)} = 5.3$, $P < 0.001$, Figure 3.6). Mean severity of seedling attack by *H. ater* ranged from 0-1.9 (below moderate attack (refer to section 3.1.2)). Fifty-five percent of seedlings, planted in the site that was most severely attacked on average by *H. ater*, were severely attacked. Ten percent and five percent of seedlings were attacked with moderate and mild severity, respectively. The remaining 30% of seedlings in this site were not attacked by *H. ater*.

Figure 3.5 Mean severity of seedling attack by *H. ater* for 32 sites planted during the winter of 1998.

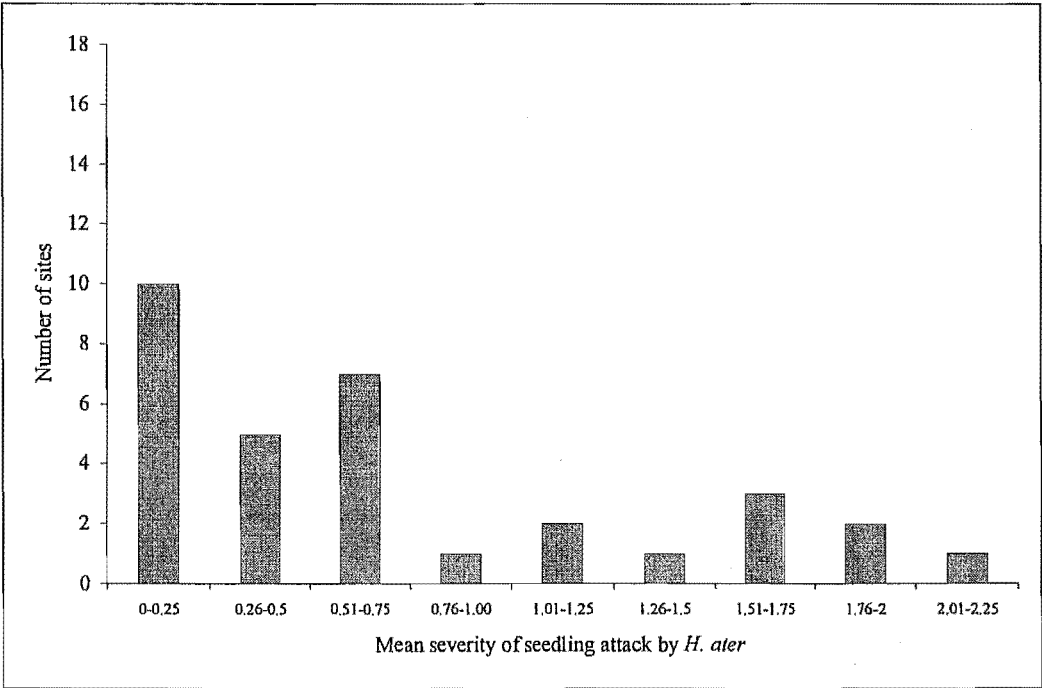
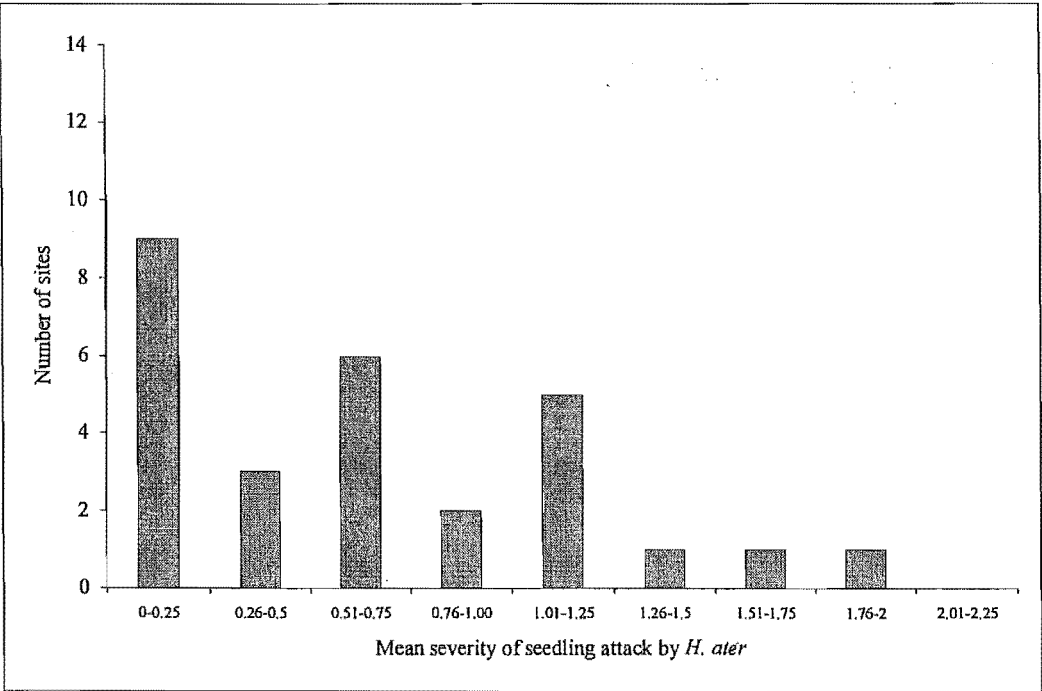


Figure 3.6 Mean severity of seedling attack by *H. ater* for 28 sites planted during the winter of 1999.



The relationship between seedling attack by *H. ater* and the harvesting history of sites

To examine the influence of the harvesting history of sites on seedling attack by *H. ater*, sites were placed into four categories based on when harvesting activities occurred. Periods of harvesting were grouped into two-monthly intervals as follows:

- 1= Harvested during the period: 1 February to 31 March
- 2= Harvested during the period: 1 December to 31 January
- 3= Harvested during the period: 1 October to 30 November
- 4= Harvested prior to 1 October

There was a significant difference in the frequency of attack on seedlings planted during winter 1998 which was associated with the time of harvesting ($\chi^2_{(3)} = 113.980$, $P < 0.001$). Seedlings planted in sites harvested during the period from 1 February to 31 March 1998 were attacked with greater frequency than seedlings planted in sites harvested at other times (Table 3.1). The amount of attack by *H. ater* decreased with increasing time between harvesting and planting (Table 3.1).

Table 3.1 The mean frequency of seedling attack by *H. ater* for sites harvested at different times (sites planted during winter 1998).

Harvesting period	Mean frequency of attack (%)	Number of seedlings sampled
1 February to 31 March 1998	65	180
1 December 1997 to 31 January 1998	35	80
1 October to 30 November 1997	29	80
Prior to 1 October 1997	15	240

Severity of attack by *H. ater* also differed between harvesting periods ($F_{(3,575)} = 57.18$, $P < 0.001$). Sites harvested during the February to March 1998 period were more likely to be attacked with greater severity than the other sites. Mean severity of seedling attack by *H. ater* decreased with increasing time between harvesting period and planting (Table 3.2). A summary of the frequency of seedling attacks by *H. ater* in sites harvested at different times (Table 3.3) shows that seedlings planted in sites harvested during the February to March period were more likely to be attacked, and that these seedlings were also attacked

with greater severity. This may have been due to a greater number of individual beetles feeding on a seedling.

Table 3.2 Results for the pairwise comparisons using Duncan’s multiple range test: Severity of seedling attack by *H. ater* for sites harvested at different times (sites planted during winter 1998).

Harvesting period	Mean severity of attack	Number of seedlings sampled
1 February to 31 March 1998	1.36 _a	180
1 December 1997 to 31 January 1998	0.55 _b	80
1 October to 30 November 1998	0.50 _b	80
Prior to 1 October 1998	0.22 _c	240

Means with the same letter are not significantly different ($\alpha = 0.05$).

Table 3.3 Frequency of seedlings attacked by *H. ater* in sites harvested at different times (sites planted during winter 1998).

Harvesting period	No attack	Mild attack	Moderate attack	Severe attack
1 February to 31 March 1998	35%	17%	24.5%	23.5%
1 December 1997 to 31 January 1998	65%	20%	10%	5%
1 October to 30 November 1997	71%	13%	11%	5%
Prior to 1 October 1997	85%	10.5%	2%	2.5%

There was a significant difference in frequency of attack on seedlings planted during winter 1999 which was associated with the time of harvesting ($\chi^2_{(3)} = 67.103$, $P < 0.001$). The results were similar to those observed for the 1998 plantings. Seedlings planted in sites harvested from 1 February to 31 March 1999 were more likely to be attacked by *H. ater* (Table 3.4). As with the 1998 plantings, seedlings were less likely to be attacked by *H. ater* with increasing time between harvesting of the previous rotation and planting of the seedlings.

Table 3.4 The frequency of seedling attack by *H. ater* in sites harvested at different times (site planted during winter 1999).

Harvesting period	Mean frequency of attack (%)	Number of seedlings sampled
1 February to 31 March 1999	65	60
1 December 1998 to 31 January 1999	45	240
1 October to 30 November 1999	22	160
Prior to 1 October 1999	13	100

The severity of seedling attack by *H. ater* differed with respect to harvesting history ($F_{(3,556)}= 18.90, P< 0.001$). Seedlings planted in sites harvested during the period of February to March 1999 were more likely to be attacked with greater severity than seedlings in other sites (Table 3.5). Mean severity of attack decreased with increasing time between harvesting and planting. A summary of the frequency of seedling attacks in sites harvested at different times (Table 3.6) shows that while seedlings planted in sites harvested from 1 February to 31 March 1999 were more likely to be attacked, seedlings were also attacked with a greater severity compared to other sites.

Table 3.5 Results for the pairwise multiple comparisons using Duncan’s multiple range test: Severity of seedling attack by *H. ater* in sites harvested at different times (site planted during winter 1999).

Harvesting period	Average severity of attack	Number of seedlings sampled
1 February to 31 March 1999	1.27 _a	60
1 December 1998 to 31 January 1999	0.90 _b	240
1 October to 30 November 1998	0.47 _c	160
Prior to 1 October 1998	0.23 _c	100

Means with the same letter are not significantly different (alpha= 0.05).

Table 3.6 Frequency of seedlings attacked by *H. ater* for the four harvesting periods (seedlings planted during winter 1999).

Harvesting period	No attack	Mild attack	Moderate attack	Severe attack
1 February to 31 March 1999	35%	27%	15%	23%
1 December 1998 to 31 January 1999	55%	14%	17%	14%
1 October to 30 November 1998	78%	5.5%	7.5%	9%
Prior to 1 October 1998	87%	6%	4%	3%

3.1.4 Discussion

While seedling death resulting from *H. ater* attack has been reported in the past in New Zealand (Zondag 1958), studies have not reported the extent of sub-lethal attack. High levels of mortality due to *H. ater* attack (i.e. greater than 50%) as described by Zondag (1958) were not observed during this study. The results show that the problems associated with seedling attack by *H. ater* are more complex than just seedling mortality. Sub-lethal *H. ater* attack may be as significant (or of greater significance) to the forestry industry in terms of loss of value, as the attack by *H. ater* that results in seedling death.

The observed seedling mortality resulting from *H. ater* attack was low (in comparison to historical reports of seedling mortality) with the exception of a small number of sites where mortality ranged from 18% to 30%. Seedling death over 90% due to *H. ater* attack has been reported (Boomsma & Adams 1943). Neumann (1987) reported that all seedlings in a 3.2 hectare area in Victoria, Australia were killed by *H. ater*. Ciesla (1988) reported seedling mortality due to *H. ater* attack as high as 70% in Chile.

Previous reports of seedling mortality due to *H. ater* attack indicated that mortality was not evenly spread throughout the forest estate (S. Downs CHH, personal communication). The results presented here confirm this. High levels of seedling mortality resulting from *H. ater* attack appeared to be confined to certain high-risk sites. Identifying high-risk sites could minimise the risks to forestry associated with *H. ater* attack.

There was no evidence that the *H. ater* attack was the sole cause of seedling mortality. While it seemed reasonable to assume that severe attack by *H. ater* was the cause of seedling mortality, other factors may have been involved. These include site, environmental or seedling characteristics. It has been suggested that *H. ater* only attacks weak seedlings and does not kill healthy trees (Bain 1973, 1980). Bain (1973, 1980) reported that seedling mortality attributable to *H. ater* was minimal. However, these results show that *H. ater* related mortality was the predominant cause of seedling mortality in the first year following planting. The mortality of undamaged seedlings was minimal compared to the mortality of seedlings that showed evidence of severe attack by *H. ater*. It seemed likely that the majority of seedlings would have survived in the absence of *H. ater* attack. This was examined in greater detail in Section 3.2. Other factors may contribute to *H. ater* feeding heavily on seedlings. Whether *H. ater* only attacks weak or less resistant seedlings was addressed in Chapter Five. If so, as suggested by Bain (1973, 1980), it is unlikely that such high levels of sub-lethal attack in growing seedlings would have been observed.

This study showed that the amount of sub-lethal seedling attack by *H. ater* was substantial. As suggested above, most cases documenting attack by *H. ater* have only described *H. ater* related seedling mortality (Clark 1932b, Boomsma & Adams 1943, Crowhurst 1969, Neumann 1987, Ciesla 1988). The frequency of sub-lethal attack and its effect on seedlings have been scarcely documented and are reported for *H. ater* attack on *P. radiata* seedlings in this study in Section 3.2 and Chapter Four. Sub-lethal attack may influence seedling growth and the invasion of seedlings by pathogens.

An important component of this study was to determine if any factors were related to high mortality and attack. If factors could be identified it would be possible to identify high-risk areas prior to planting. This would allow management decisions to be made regarding intended plantings. It would then be possible to treat 'at risk' seedlings or to avoid planting certain areas. Leather *et al* (1999) discuss attempts to identify site factors that predispose seedlings to attack by *Hylobius abietis*. Studies have investigated factors that effect the suitability of the breeding site, the rate of *H. abietis* development, tree planting and weevil-seedling interactions (Leather *et al* 1999). Despite the large amount of research that has addressed this problem, high-resolution predictive models have not been satisfactory. However, models which operate at lower levels of resolution, have been more

successful when used to forecast 'at risk' areas (Leather *et al* 1999). While resources limited this study, it was still possible to identify some factors that influence the risk of seedlings to *H. ater* attack.

The time of year that sites are harvested appears from the data to be an important factor in determining whether seedlings are likely to be attacked by *H. ater*. The results showed that sites harvested during February and March, and planted the following winter, were at the greatest risk from *H. ater* attack.

The relationship between harvesting history and the likelihood of attack by *H. ater* can be related to the flight activity and life history of *H. ater* (as discussed in Chapter Two). The stumps colonised early in the summer period were degraded prior to the planting of seedlings the following winter. Observations made during studies of the life cycles of *H. ater* and *H. ligniperda* in Chapter Two indicated that most, or often all larval food material was consumed during the first generation of beetle populations in stumps. Following this, stumps were unsuitable for re-colonisation by breeding adults. While there may occasionally have been an opportunity for a second generation to breed in stumps, any emerging populations were likely to be greatly reduced.

The flight activity of *H. ater* and *H. ligniperda* studied in Chapter Two demonstrated that *H. ligniperda* was the dominant species for most of the summer. The majority of sites harvested during the period from October to February were colonised predominantly by *H. ligniperda*. There was *H. ater* activity during this period and *H. ater* adults colonised these sites in low numbers. The main period of *H. ater* flight activity was during autumn (March to April). During this period *H. ater* was the dominant species colonising recently harvested sites.

Seedling attacks result from the maturation feeding of emerging *H. ater* adults. For this to occur, seedlings have to be planted prior to the emergence of beetles. Currently, operational practices are to plant seedlings during the winter months. The only sites where reasonable numbers of larvae are still present in stumps at the time of planting are those that were colonised by *H. ater* during the later part of summer. Larval development could not be completed by the end of summer and *H. ater* overwintered as larvae. Development of these larvae was completed during the following spring and summer months. Emerging

beetles were able to begin maturation feeding on seedlings that were planted while they were developing as larvae in the surrounding stumps during winter. There is no evidence that overwintering *H. ligniperda* larval populations are a threat to seedlings in New Zealand (Bain 1977, personal observations). It is the sites that were colonised by *H. ater* at the end of summer that sustained the largest overwintering *H. ater* larval populations. Hence seedlings planted in sites harvested during February and March were at the greatest risk from *H. ater* attack.

Seedlings not planted in sites with overwintering populations of *H. ater* larvae were unlikely to be attacked by *H. ater*. Seedlings that did not suffer *H. ater* damage when planted in sites which had remained fallow for extended periods demonstrates the efficacy of one of the oldest cultural techniques of damage reduction. *H. ater* emerged from stumps and began maturation feeding on the available food material (i.e. seedlings). If food material was unavailable (i.e. no seedlings had been planted) the newly emerged *H. ater* adults likely dispersed from the site and searched for an alternative food source. There was no indication that emerging *H. ater* adults attempted to re-invade stumps at the sites they emerged in. Seedlings planted in older sites were not attacked despite a site with emerging beetles being in close proximity. These sites may have been directly across a road or in the same compartment. Emerging adults appeared unable to detect seedlings unless they emerged among them. As the stumps in such sites would have been unlikely to be attractive to adult beetles, the volatiles attracting beetles to these sites were likely to be minimal. Once emerging beetles had initiated flight activity they would have searched for volatiles from recently harvested stumps (or similar material). Older sites would have been a relatively poor source of volatiles, compared with volatiles released from recently harvested areas.

This emergence and host searching behaviour of *H. ater* in New Zealand differs from that of *Hylobius abietis* in Britain and Europe. Adult *H. abietis* will come from outside planted areas to feed on seedlings (Leather *et al* 1999). Predicting the risks associated with *H. abietis* is more complicated than predicting the size of populations emerging from brood material in planted sites.

Biological and environmental factors are likely to influence the extent to which seedlings are attacked, by affecting the size of the adult *H. ater* population that emerges from stumps

and attacks seedlings during maturation feeding. These may include the relative abundance of *H. ater* and *H. ligniperda* larval populations in a site. The factors that may influence larval survival include competition for resources (both inter- and intra-specific competition), amount of food source available (stump size and condition), climate, aspect, slope, topography, soil type and site preparation prior to planting. Assessing which of these factors may be important would require a substantial research effort, quantifying the relative numbers of colonising adults, subsequent larval populations and the resulting emerging beetle populations, and the frequency and severity of attacks on seedlings. A study such as this was beyond the scope of this thesis research.

The factors that influence *H. ater* populations (and the subsequent amount of seedling damage) may also influence seedling health, and therefore the ability of a seedling to resist attack. Isolating one factor from another may not be possible given the potential interaction between factors. If environmental factors were identified as having an influence on either the populations of emerging *H. ater* adults or the ability of seedlings to resist attack, these could probably not be altered within an operational forestry environment. If high-risk sites are identified treatment should be initiated regardless of whether site factors may influence *H. ater* numbers or seedling response. It is possible that the genetic characteristics of seedlings may influence how seedlings respond to attack by *H. ater*. These were more easily studied and are the subject of Chapter Five.

While seedling mortality of more than 30% resulting from *H. ater* attack was not observed in this study, the results show that *H. ater* seedling mortality is a significant problem to *P. radiata* culture in New Zealand. The major cause of seedling death was from *H. ater* maturation feeding during the first year after planting. While levels of seedling mortality were generally low, the frequency of sub-lethal attack on seedlings by *H. ater* was higher. Particularly severe seedling attack by *H. ater* is likely to have some effect on seedlings, if not by directly influencing growth, then by creating a wound that is a potential site for invasion by pathogens. The harvesting history of a site was found to be the most important factor when predicting seedling attack by *H. ater*. 'High-risk' sites were those that were harvested at the end of summer, and planted the following winter. Being able to identify high-risk sites means operational strategies to manage the risk to *H. ater* may be implemented prior to planting. Understanding the implications of *H. ater* attack on

seedlings (particularly sub-lethal attack) formed the basis of the following research in this project.

3.2 The influence of sub-lethal attack by *H. ater* on the growth of seedlings

3.2.1 Introduction

One potential effect of sub-lethal attack is that it may result in reduced growth increment. Moderate attack by the bark weevil *Hylobius abietis* has been suggested to inhibit the growth of seedlings some years after planting (Leather *et al* 1999). If attacked seedlings do show reduced growth rates; this may have serious implications for the selection of suitable trees during thinning and other operational activities. Seedlings attacked by *H. ater* that fail to establish successfully in the first year after planting may be over grown by seedlings that were not attacked.

Marshal suSCon® has been shown to confer resistance to seedlings for three years against bark beetle attack of conifers in Europe (Lemperière & Julien 1989, Mrlina *et al* 1994, Heritage *et al* 1997a, Leather *et al* 1999). Marshal suSCon® contains a carbamate insecticide widely used in agriculture against a broad spectrum of insect pests (Heritage *et al* 1997a). Marshal suSCon® is a controlled-release carbosulfan insecticide (10% carbosulfan).

A single application of Marshal suSCon® at planting time kills bark beetles when a lethal dose is ingested. Some damage to treated seedlings may be expected (Heritage *et al* 1997a). Following application to the soil, Marshal suSCon® is absorbed by plant roots and transported to the stem and needles. Carbosulfan is only slightly soluble in water and is therefore fairly immobile in the soil (Heritage *et al* 1997a). It does break down very rapidly, lasting only a few days in both soil and plant tissues (Heritage *et al* 1997a).

The granule consists of a plastic matrix structure that prevents the insecticide being broken down and controls its release into the surrounding soil (Heritage *et al* 1997a). This ensures

that carbosulfan is maintained around the roots, with the insecticide constantly being broken down and replaced (Heritage *et al* 1997a).

As of 2000, Marshal suSCon® is unavailable for use in New Zealand. These experiments provided the first opportunity to test the insecticide in New Zealand second rotation *P. radiata* forests against *H. ater* attack. The results may be used for registration purposes so that likely benefits may be captured in operational use.

The primary objective of this study was to test the efficacy of Marshal suSCon® against *H. ater* attack and related mortality. Any phytotoxic effects of the insecticide were evaluated by assessing the relative growth rates of the seedlings in this study. Atkinson and Govender (1997) observed that carbosulfan CR granules were phytotoxic to *Pinus patula* in South Africa when applied against *Hylastes angustatus* attack.

Testing the efficacy of Marshal suSCon® provided the opportunity to establish a chemical enclosure trial. Protecting only some seedlings from attack by *H. ater* meant that the influence of *H. ater* attack on seedling growth could be addressed.

The objectives were to:

- Determine whether Marshal suSCon® protected seedlings from attack by *H. ater*
- Determine whether sub-lethal seedling attack by *H. ater* had an influence on the growth of seedlings

3.2.2 Methods

Six sites were selected from second rotation *P. radiata* forests following visual bark inspections, based on a line transect through prospective sites. Three forestry companies provide sites. They were Fletcher Challenge Forests (Kaingaroa Forest), Carter Holt Harvey Forests (Kinleith Forest) and Rayonier New Zealand (Te Wera Forest) (Figure 2.1). Kinleith and Kaingaroa Forests were described in section 2.1.2.

Te Wera Forest is in the eastern hill country of Taranaki and consists of 2 580 hectares of *P. radiata* plantation forest. The soils are a complex of Whangamomoa steeppland soils and

New Plymouth hill soils. Whangamomoa steepland soils are found on steeper slopes where volcanic ash is thin (New Zealand Forest Corp. 1989). New Plymouth hill soils are found on less steep slopes where volcanic ash deposits (Egmont Ash) are present (New Zealand Forest Corp. 1989).

Annual rainfall is approximately 1 800 mm (Thompson 1981). Winter is the wettest season; with the months of May to July each having 10% of the annual rainfall, while the driest month is February or March (Thompson 1981). Dry spells and droughts do occur occasionally. Periods of high rainfall are more common. The prevailing winds are from the southeast and winds of gale force are rare (Thompson 1981). The area is characterised by mild temperatures during the summer (mean afternoon summer temperature 20°C) and much cooler temperatures during the winter (especially at night). January and February are the warmest months, with mean daily temperatures of 16°C (Thompson 1981). The mean daily temperature during the winter months is approximately 7°C and frosts occur regularly (Thompson 1981). Thunder and hail are common, while snow is rare (Thompson 1981). There are 2 000-2 100 hours of bright sunshine per year (Thompson 1981).

Each of the three companies contributed two sites and provided seedlings and planting crew to establish the trials. Sites were selected if satisfactory populations of *H. ater* larvae were detected during inspections prior to the establishment of the trials. Crop Care Holdings Ltd supplied Marshal suSCon®.

The trials were laid out in a randomised complete block design. Each trial consisted of seven replicated blocks containing three treatments. Blocks were separated from each other by two rows of buffer trees. The treatments were as follows:

- No Marshal suSCon® (control)
- 10g Marshal suSCon®
- 15g Marshal suSCon®

Each treatment consisted of one row of 100 trees. Each site contained 2 100 seedlings planted in the three treatments, with an additional 360 seedlings planted in buffer rows

(buffer rows were planted with greater spacing between seedlings). In total 12 600 seedlings were planted in the trials.

Contractors and trainees planted the trials with supervision by a company representative and the author. Normal 'operational' procedures were followed during planting in order to emulate replanting techniques utilised in the forests.

Marshal suSCon® was applied to the roots of the trees after they were placed in the hole and before the tree was 'firmed' in. Two people were required to plant those treatments where the application of the chemical was required. The chemical was applied using a measuring cup filled from a plastic bucket. One person digging the hole and positioning the seedling, with a second person applying the required dose and firming in the seedling, was the best method of planting and applying the chemical.

The sixth site, RNZ2 was abandoned following assessment six months after planting. Goats had caused considerable damage. Approximately half the seedlings in the site had been eaten down to ground level. As the Rayonier New Zealand sites were considerably further from the other sites, the growth measurements in the site RNZ1 were abandoned. With the large number of seedlings planted in the remaining four sites, sufficient data was gathered to adequately investigate any effect of *H. ater* attack on the growth of seedlings.

Sampling periods

The sampling dates for the study were as follows:

Site	Baseline	1 st sample	2 nd sample	3 rd sample	Final sample
CHH1	12/9/97	1/2/98	23/8/98	6/2/99	7/9/99
CHH2	2/9/97	30/1/98	21/8/98	7/2/99	25/8/99
FCF1	15/9/97	3/2/98	13/7/98	13/2/99	23/8/99
FCF2	15/9/97	2/2/98	14/7/98	14/2/99	24/8/99
RNZ1	6/9/97	28/1/98	21/7/98		2/9/99

Sites were sampled for evidence of *H. ater* induced seedling mortality, attack and growth as follows:

Mortality

A mortality survey was undertaken every six months. All dead seedlings in each treatment were removed from the ground and inspected for evidence of *H. ater* attack. Mortality was attributed to *H. ater* only if severe attack was evident on the dead seedling.

Attack

Every second seedling was removed, until 20 seedlings had been removed from each row, one and two years after planting. For each seedling sampled, evidence and severity of attack were recorded. The severity of any attack was recorded and described in section 3.1.2.

Growth

The potential influence of Marshal suSCon® on the growth of seedlings was determined by measuring the height and diameter growth of a sample of seedlings from each treatment. Measurements were made one month following planting (Baseline data), then at six month intervals until seedlings were two years old. Seedlings in the four sites CHH1, CHH2, FCF1 and FCF2 were measured.

Every second seedling was measured in each treatment. This sample size was considered large enough to detect differences in relative growth rates for the different treatments. In total, 4 200 seedlings were measured during each sampling period.

Seedling height was measured from the base of the stem to the top of the crown. Seedling diameter was measured as close to the base of the stem as possible, using digital callipers. Care was taken to ensure that no needles were caught in the calliper 'jaws' and included in the stem diameter measurement.

Data collected on seedling mortality and attack by *H. ater* were analysed using chi-square tests, to investigate differences between treatments, with the statistical programme SAS (PROC FREQ, Version 6.12 for Windows, SAS Institute 1996). Analyses of variance (ANOVA) were used to compare the severity of *H. ater* attack between the three

treatments and the influence of treatment on the growth of seedlings, using the statistical package SAS (PROC GLM, Version 6.12 for Windows, SAS Institute 1996). Pair-wise multiple comparisons were conducted using Duncan's multiple range test to determine the nature of the differences detected by ANOVA

3.2.3 Results

The efficacy of Marshal suSCon® against *Hylastes ater* attack

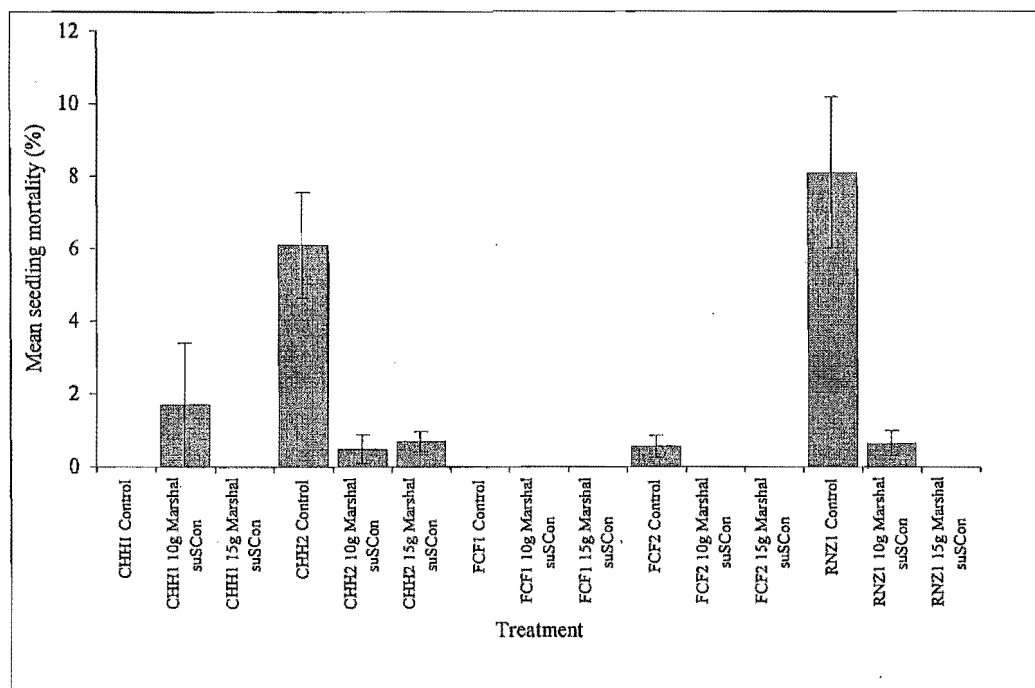
Mortality as a result of *H. ater* attack

All seedlings that died after *H. ater* attack did so within the first year of planting. There was no *H. ater* related mortality following the second sample. Mean mortality was significantly different between treatments ($F_{(2,92)} = 10.97$, $P < 0.001$, Figure 3.7). Untreated seedlings were killed more often by *H. ater* than seedlings treated with Marshal suSCon® (Table 3.7). Seedling mortality as a result of *H. ater* feeding attacks was highest in site RNZ1. In site RNZ1, the control treatments had a mean mortality of 8.1%. Mean mortality for the 10g Marshal suSCon® treatments at the RNZ1 site was 0.7%, while no seedlings treated with 15g Marshal suSCon® were killed by *H. ater*. Mortality due to *H. ater* attack was not significantly different between seedlings treated with 10g and 15g Marshal suSCon® (Table 3.7). Mortality did not differ between blocks ($F_{(6,92)} = 0.32$, $P > 0.05$), but did differ between sites ($F_{(4,92)} = 5.24$, $P < 0.001$). This indicates that there were differences in *H. ater* populations between sites.

Table 3.7 Results for the pairwise comparisons using Duncan's multiple range test: Mean seedling mortality resulting from *H. ater* attack.

Treatment	Mean Mortality (%)	Number of rows
Control (no treatment)	2.97 _a	35
10g Marshal suSCon®	0.57 _b	35
15g Marshal suSCon®	0.15 _b	35

Means with the same letter are not significantly different ($\alpha = 0.05$).

Figure 3.7 Mean mortality due to *H. ater* attack one year after planting.

Sub-lethal feeding attacks on seedlings by *H. ater*

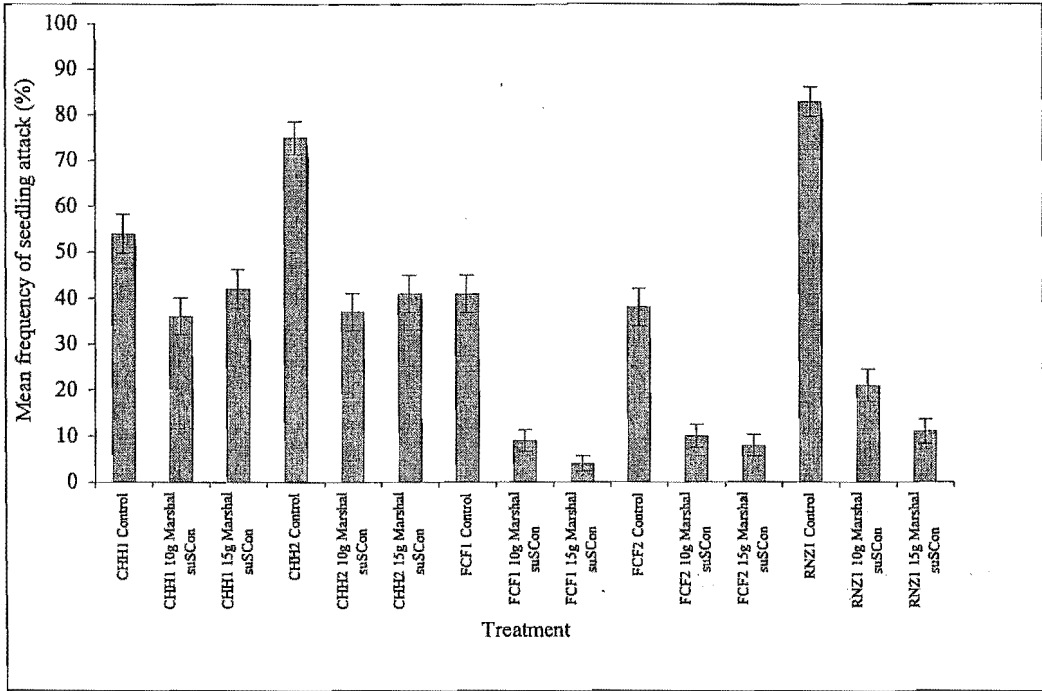
i) One year after planting

Untreated seedlings were attacked with greater frequency than seedlings treated with Marshal suSCon® ($X^2_{(2)} = 271.783$, $P < 0.001$, Table 3.8, Figure 3.8). On average, 83% of untreated seedlings in RNZ1 showed evidence of *H. ater* attack. Mean attack on untreated seedlings in other sites ranged from 38% (FCF2) to 75% (CHH1). Mean attack on seedlings treated with 10g Marshal suSCon® ranged from 8.5% (FCF1) to 37% (CHH2). Attacks on seedlings treated with 15g Marshal suSCon® ranged from 4% (FCF1) to 42% (CHH1). There was no significant difference in the proportion of seedlings attacked between the Marshal suSCon® treatments (10g & 15g) ($X^2_{(1)} = 0.353$, $P > 0.05$, Figure 3.8). There was a significant difference in the number of seedlings attacked between sites ($X^2_{(4)} = 169.778$, $P < 0.001$). This indicates that there were differences in *H. ater* populations between sites.

Table 3.8 Mean frequency of seedling attack by *H. ater* after one year.

Treatment	Mean attack (%)	Number of seedlings sampled
Control (no treatment)	58.2	699
10g Marshal suSCon®	22.7	700
15g Marshal suSCon®	21.4	701

Figure 3.8 Mean frequency of seedling attack by *H. ater* after one year.



ii) Two years after planting

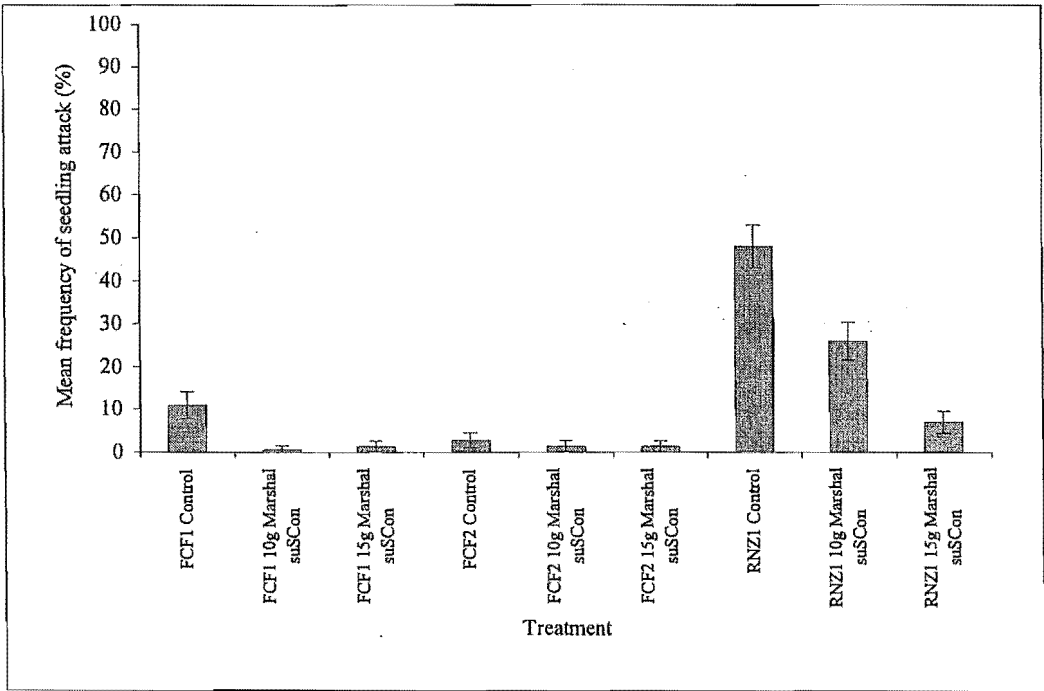
Untreated seedlings still showed evidence of a higher frequency of attack by *H. ater* than treated seedlings in the second year after planting ($X^2_{(2)} = 52.103$, $P < 0.001$, Table 3.9, Figure 3.9). However, the number of seedlings attacked by *H. ater* was less compared with the first sample one year after planting. On average, 18% of untreated seedlings showed evidence of attack after two years, while seedlings treated with 10g and 15g of Marshal suSCon® showed a mean frequency of attack of 8% and 3% respectively (Table 3.9, Figure 3.9). Mean attack on untreated seedlings the previous year was 58%. Although feeding attacks by *H. ater* on treated seedlings were less common; there was a relationship

between the dose of Marshal suSCon® and the frequency of damage. The higher dose treatment (15g) showed less damage after two years ($\chi^2_{(1)}= 8.55, P< 0.01$, Figure 3.9). There was a significant difference in the number of seedlings attacked between sites ($\chi^2_{(2)}= 147.505, P< 0.001$).

Table 3.9 Mean frequency of seedling attack by *H. ater* after two years.

Treatment	Mean attack (%)	Number of seedlings sampled
Control (no treatment)	17.9	380
10g Marshal suSCon®	7.6	380
15g Marshal suSCon®	2.9	380

Figure 3.9 Mean frequency of seedling attack by *H. ater* after two years.



Severity of sub-lethal feeding attacks on seedlings

i) One year after planting

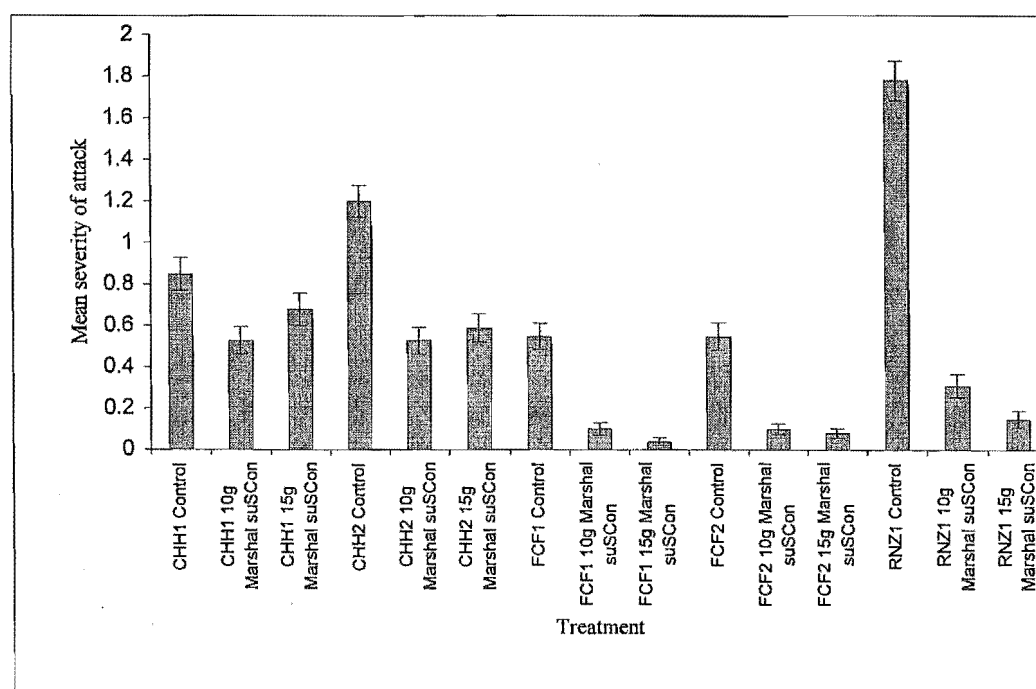
Untreated seedlings were attacked with greater severity than treated seedlings ($F_{(2,2087)} = 186.70$, $P < 0.001$, Table 3.10, Figure 3.10). Untreated seedlings in site RNZ1 suffered the most severe damage with a mean severity of attack of 1.78 (Moderate). Mean damage levels for seedlings treated with 10g Marshal suSCon® ranged from 0.1 (FCF1, FCF2) to 0.52 (CHH1, CHH2). Seedlings treated with 15g Marshal suSCon® had a mean severity of attack from 0.04 (FCF1) to 0.67 (CHH1) after one year. There was no significant difference between damage levels after treatment with 10g or 15g Marshal suSCon (Table 3.10, Figure 3.10). There was a significant difference in damage levels between blocks ($F_{(2,2087)} = 3.04$, $P < 0.01$) and sites ($F_{(4,2087)} = 55.26$, $P < 0.001$).

Table 3.10 Results for the pairwise comparisons using Duncan's multiple range test: Mean severity of seedling attack by *H. ater* after one year.

Treatment	Mean severity of attack	Number of seedlings sampled
Control (no treatment)	0.99 _a	699
10g Marshal suSCon®	0.31 _b	700
15g Marshal suSCon®	0.31 _b	701

Means with the same letter are not significantly different ($\alpha = 0.05$).

Figure 3.10 Mean severity of seedling attack by *H. ater* after one year.



ii) Two years after planting

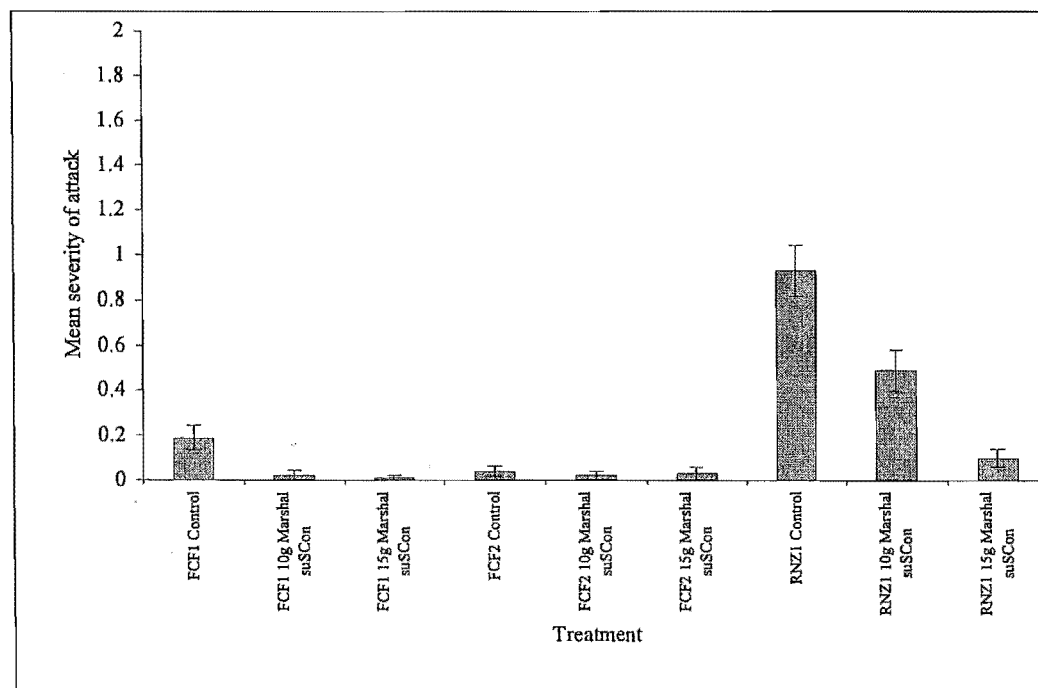
Untreated seedlings had significantly higher levels of damage, than seedlings treated with Marshal suSCon®, in the second year after planting ($F_{(2,1129)} = 28.23$, $P < 0.001$, Table 3.11, Figure 3.11). The mean severity of seedling attack was lower after two years (0.33) compared with one year after planting (0.99), and untreated seedlings still suffered the greatest damage. Damage levels in the FCF sites were very low when compared with the results after one year. Seedlings treated with the lower dose of 10g Marshal suSCon® suffered more damage than the higher dose treatment (15g Marshal suSCon®) (Table 3.11). There was a significant difference in the severity of damage to seedlings between blocks ($F_{(6,1129)} = 7.97$, $P < 0.001$) and sites ($F_{(2,1129)} = 75.03$, $P < 0.001$).

Table 3.11 Results for pairwise comparisons using Duncan's multiple range test: Mean severity of seedling attack by *H. ater* after two years.

Treatment	Mean severity of attack	Number of seedlings sampled
Control (no treatment)	0.33 _a	380
10g Marshal suSCon®	0.15 _b	380
15g Marshal suSCon®	0.04 _c	380

Means with the same letter are not significantly different ($\alpha = 0.05$).

Figure 3.11 Mean severity of seedling attack by *H. ater* after two years.



The influence of *H. ater* attack on seedling growth

The growth (height and diameter) of seedlings was sampled in four sites (FCF 1 and 2 and CHH 1 and 2). A baseline sample was made following the planting of seedlings. Four samples were made at six-monthly intervals.

i) Baseline sample

There was not a significant difference in height between seedlings at the time of planting ($F_{(2,4184)} = 0.17$, $P > 0.05$). There were significant differences in height between blocks ($F_{(6,4184)} = 2.27$, $P < 0.05$) and sites ($F_{(3,4184)} = 187.25$, $P < 0.001$). Seedling diameter did differ between treatments ($F_{(2,4184)} = 4.70$, $P < 0.01$) and sites ($F_{(3,4184)} = 98.57$, $P < 0.001$). Seedlings treated with 15g Marshal suSCon® were larger in diameter than seedlings treated with 10g Marshal suSCon® and untreated (control) seedlings (Table 3.12). The diameter of seedlings was not significantly different between blocks ($F_{(3,4184)} = 1.08$, $P > 0.05$).

Table 3.12 Results for the pairwise comparisons using Duncan's multiple range test: Mean seedling diameter after planting.

Treatment	Mean Diameter (mm)	Number of seedlings sampled
15g Marshal suSCon®	6.4 _a	1400
10g Marshal suSCon®	6.3 _b	1399
Control (no treatment)	6.2 _b	1397

Means with the same letter are not significantly different ($\alpha = 0.05$).

The differences in seedling diameter between treatments at the time of planting were likely to be due to planting differences. Seedling material may have differed between sites and was a source of variation at the time of planting. However, an assumption was made that the seedling material planted in each site was consistent. It had been assumed that differences in height and diameter of seedlings at planting would be evenly distributed within sites due to the randomised design of the trials.

The seedlings planted in each site were from the same seedling stock and were assumed to have the same vigour. An assumption was made that these differences would be unlikely

to influence the future growth rates of seedlings and therefore not influence the results of the study. In addition, the study was designed to account for the sources of variation discussed here.

ii) Sample 1: six months after planting

Six months after planting there was not a significant difference in seedling height between treatments ($F_{(2,4188)} = 3.80$, $P < 0.05$). There were significant differences in seedling growth between blocks ($F_{(6,4188)} = 5.80$, $P < 0.001$) and sites ($F_{(3,4188)} = 113.51$, $P < 0.001$). Six months after planting there was a significant difference in the diameter of seedlings between treatments ($F_{(2,4188)} = 3.80$, $P < 0.05$). Seedlings treated with 10g Marshal suSCon® had thinner stems compared with seedlings in the control and 15g Marshal suSCon® treatments (Table 3.13). There were significant differences in the diameter of seedlings with respect to block ($F_{(6,4188)} = 3.56$, $P < 0.01$) and site ($F_{(3,4188)} = 124.17$, $P < 0.001$).

Table 3.13 Results for the pairwise comparisons using Duncan's multiple range test: Mean seedling diameter after six months.

Treatment	Mean Diameter (mm)	Number of seedlings sampled
Control (no treatment)	7.9 _a	1400
15g Marshal suSCon®	7.9 _a	1400
10g Marshal suSCon®	7.7 _b	1400

Means with the same letter are not significantly different ($\alpha = 0.05$).

iii) Sample 2: one year after planting

One year after planting there was a significant difference in seedling height between treatments ($F_{(2,4188)} = 3.50$, $P < 0.05$). Seedlings treated with 15g Marshal suSCon® were significantly taller than seedlings treated with 10g Marshal suSCon® (Table 3.14). Untreated seedlings were not significantly different from either the 15g or 10g Marshal suSCon® treatments (Table 3.14). Seedling height was significantly different between blocks ($F_{(6,4188)} = 4.78$, $P < 0.001$) and sites ($F_{(3,4188)} = 775.15$, $P < 0.01$). Seedling diameter was not significantly different between treatments ($F_{(2,4188)} = 2.34$, $P > 0.05$). The diameter

of seedlings was different between blocks ($F_{(6,4188)} = 7.06$, $P < 0.01$) and sites ($F_{(3,4188)} = 795.96$, $P < 0.01$).

Table 3.14 Results for pairwise comparisons using Duncan's multiple range test: Mean seedling height after one year.

Treatment	Mean Height (cm)	Number of seedlings sampled
Control (no treatment)	57.3 _a	1400
15g Marshal suSCon®	56.7 _{ab}	1400
10g Marshal suSCon®	56.0 _b	1400

Means with the same letter are not significantly different ($\alpha = 0.05$).

iv) Sample 3: 18 months after planting

Seedling height was significantly different between treatments eighteen months after planting ($F_{(2,4172)} = 7.63$, $P < 0.01$). Untreated seedlings were significantly taller than treated seedlings (Table 3.15). Height was not significantly different between blocks at this time ($F_{(6,4172)} = 1.55$, $P > 0.05$), although the height of seedlings did differ between sites ($F_{(3,4172)} = 1201.29$, $P < 0.001$).

Table 3.15 Results for the pairwise comparisons using Duncan's multiple range test: Mean seedling height after eighteen months.

Treatment	Mean Height (cm)	Number of seedlings
Control (no treatment)	125.4 _a	1400
15g Marshal suSCon®	122.6 _b	1399
10g Marshal suSCon®	121.7 _b	1385

Means with the same letter are not significantly different ($\alpha = 0.05$).

Seedling diameter was significantly different between treatments 18 months after planting ($F_{(2,4172)} = 9.18$, $P < 0.001$). At this time, untreated seedlings were significantly thicker than those seedlings treated with 10g and 15g Marshal suSCon® (Table 3.16). There were significant differences in diameter between blocks ($F_{(6,4172)} = 3.03$, $P < 0.01$) and sites ($F_{(3,4172)} = 1099.97$, $P < 0.001$).

Table 3.16 Results for the pairwise comparisons using Duncan's multiple range test: Mean seedling diameter after eighteen months.

Treatment	Mean Diameter (mm)	Number of seedlings sampled
Control (no treatment)	29.51 _a	1400
15g Marshal suSCon®	28.75 _b	1399
10g Marshal suSCon®	28.45 _b	1385

Means with the same letter are not significantly different ($\alpha=0.05$).

v) Sample 4: Final sample, two years after planting

The mean growth of seedlings (height and diameter) over the period of the study are presented in Figures 3.12 to 3.19 and summarised below. Growth differences among the sites were substantial over a relatively short period of time.

Growth of seedlings to two years of age: CHH1

The height and diameter growth of seedlings planted was similar between treatments until 18 months following planting (Figures 3.12 & 3.13). Following this, seedlings treated with 15g Marshal suSCon® grew less than seedlings treated with 10g Marshal suSCon® and untreated seedlings. The slower growth of seedlings treated with 15g Marshal suSCon® in this site may have been due to site effects (e.g. area of soil compaction).

Growth of seedlings to two years of age: CHH2

The growth of seedlings from the three treatments was not different after planting (Figures 3.14 & 3.15). There was not a significant difference in growth between treatments during the trial period.

Growth of seedlings to two years of age: FCF1

The height of seedlings was not different between treatments following planting (Figure 3.16). However, the mean diameter of untreated seedlings at this time was less than the treated seedlings (Figure 3.17). During the remainder of the trial there were no growth differences detected between the three treatments.

Growth of seedlings to two years of age: FCF2

The height of seedlings treated with 10g Marshal suSCon® was less than seedlings from the other two treatments following planting (Figure 3.18). There was no difference in seedling diameter between treatments (Figure 3.19). During the trial period, seedlings treated with 15g Marshal suSCon® grew faster than seedlings treated with 10g Marshal suSCon® and untreated seedlings.

The final height of the seedlings was different between the four trial sites after two years. Seedlings planted in site CHH2 were the tallest and were approximately 2m tall on average. Those seedlings planted in site FCF2 were approximately 1.7m tall on average. The height of seedlings planted in site CHH1 averaged approximately 1.5m. Seedlings in site FCF1 were the slowest growing and were 1.2m tall on average after two years.

These differences in growth were likely to be due to differences in site factors and the seedling types planted in the different trial sites. The results show that seedlings grew mostly during the second year after planting. The first year after planting is likely to be the period when seedlings become established. It is during the establishment period that seedlings are most vulnerable to attack by *H. ater* (Smalley *et al* 1993).

Figure 3.12 Mean height growth of seedlings over the two-year period: CHH 1

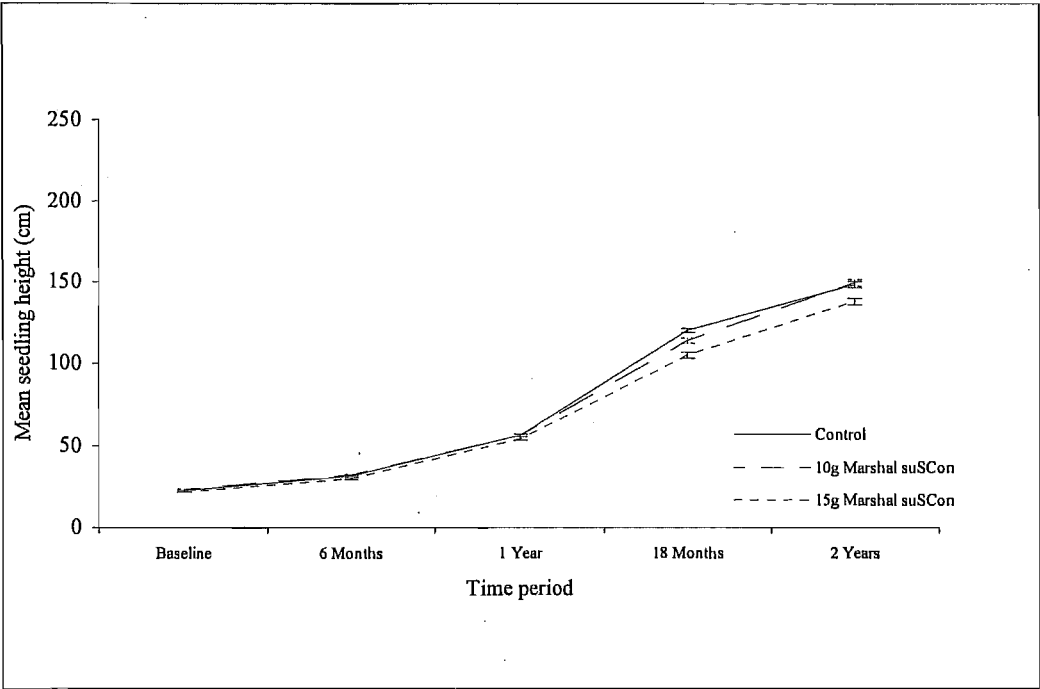


Figure 3.13 Mean diameter growth of seedlings over the two-year period: CHH 1

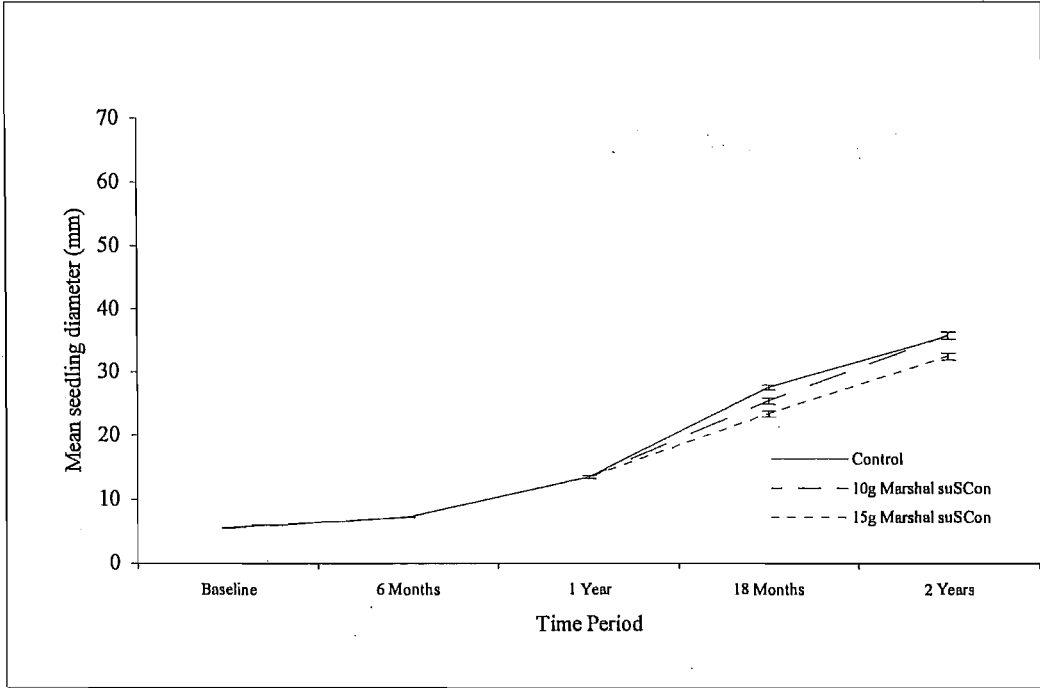


Figure 3.14 Mean height growth of seedlings over the two-year period: CHH 2

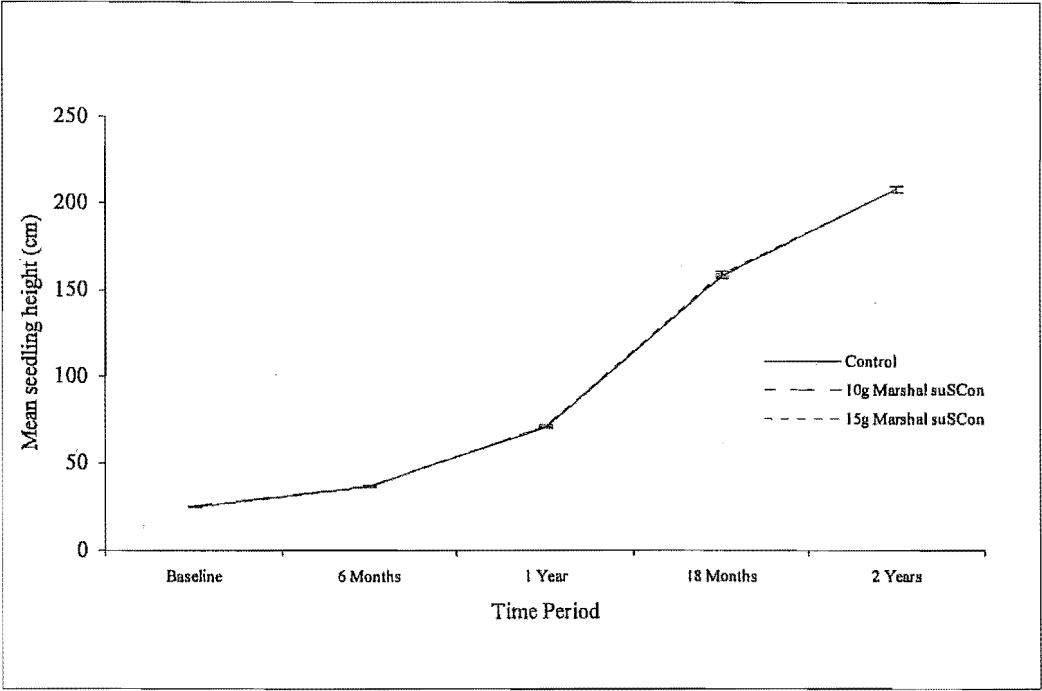


Figure 3.15 Mean diameter growth of seedlings over the two-year period: CHH 2

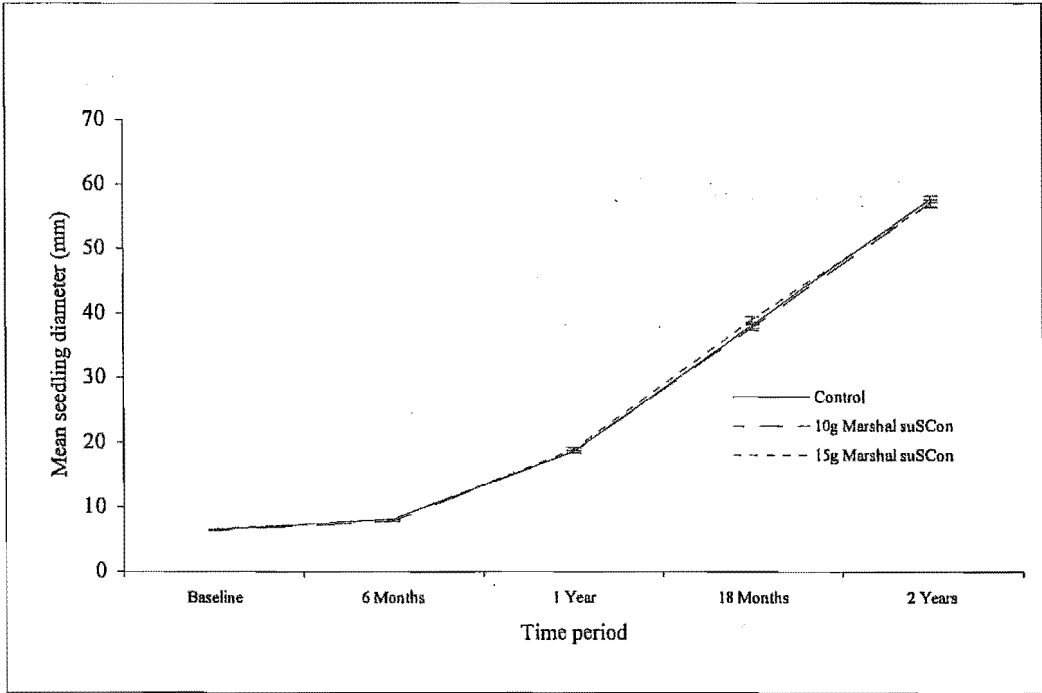


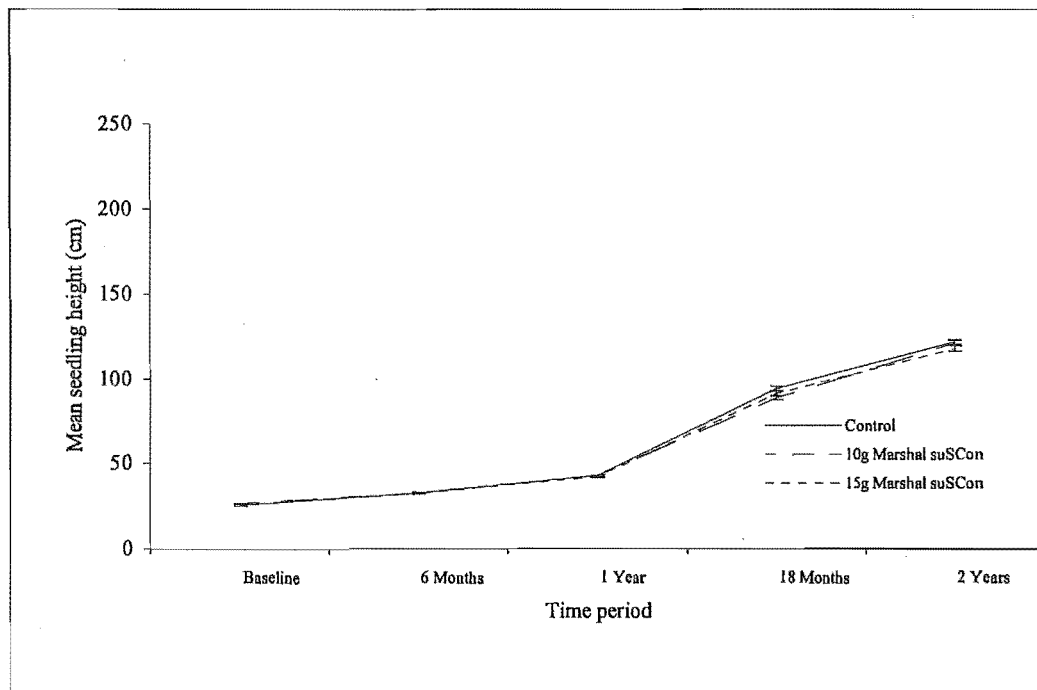
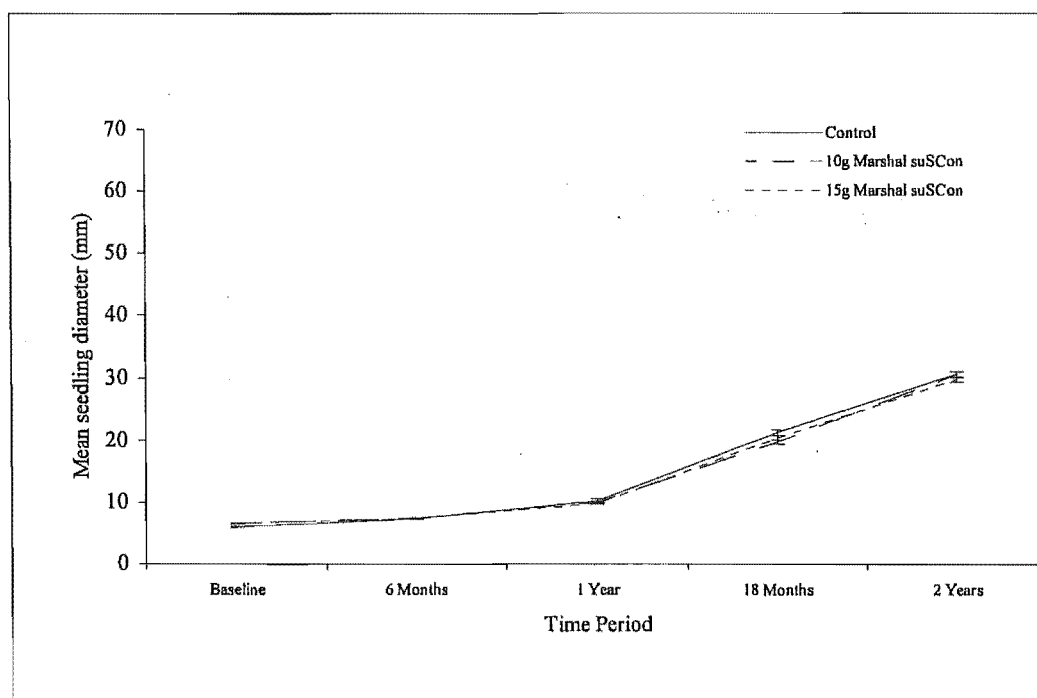
Figure 3.16 Mean height growth of seedlings over the two-year period: FCF 1**Figure 3.17** Mean diameter growth of seedlings over the two-year period: FCF 1

Figure 3.18 Mean height growth of seedlings over the two-year period: FCF 2

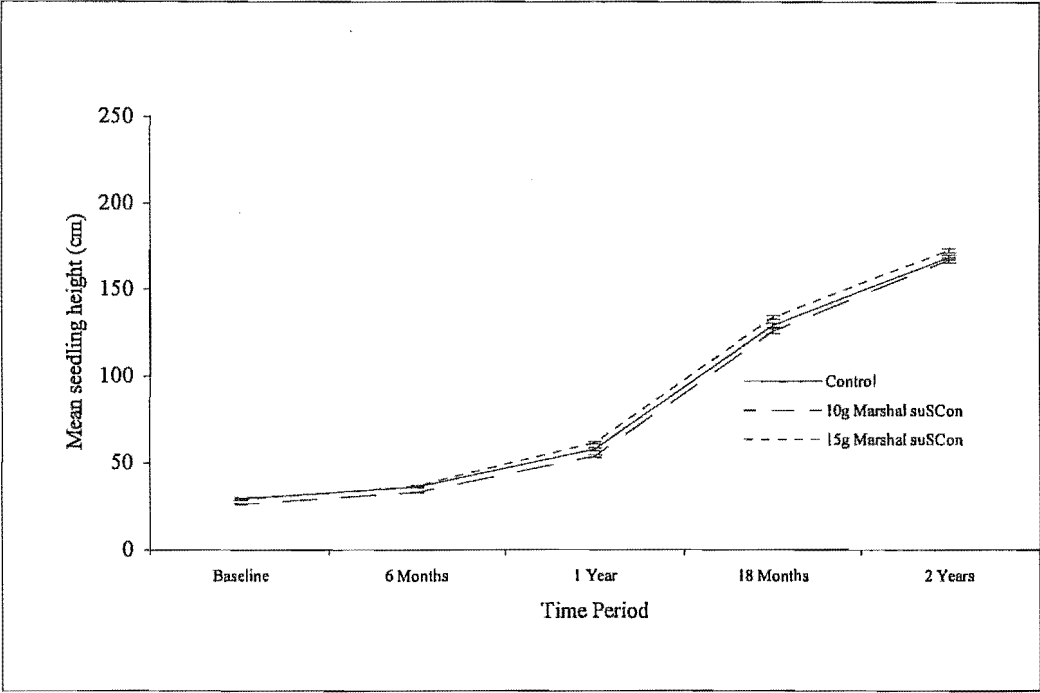
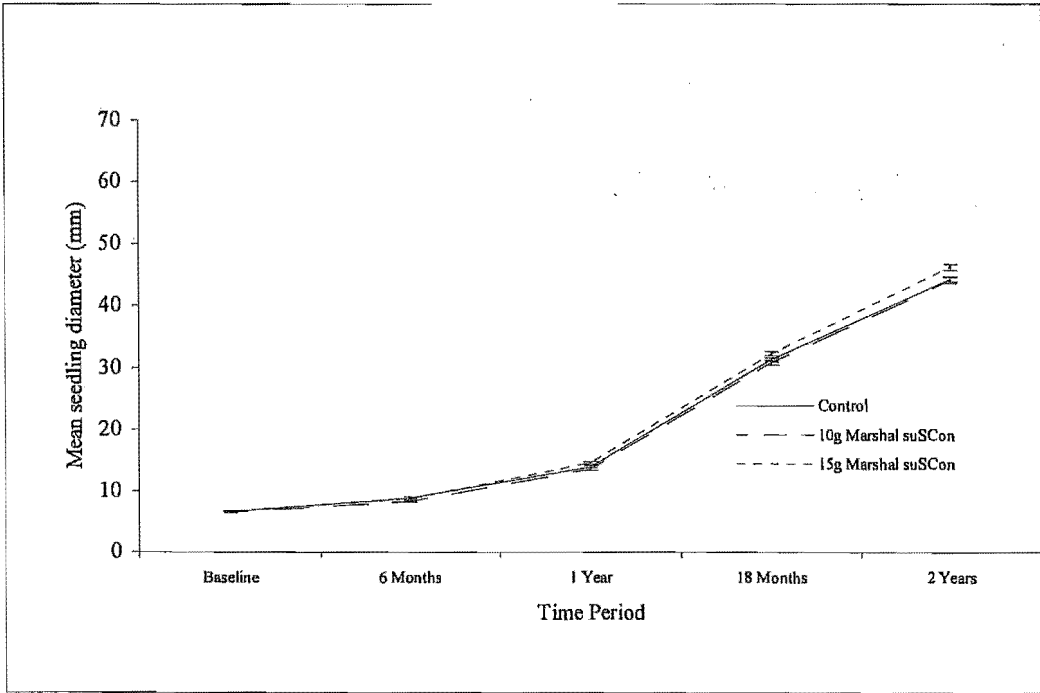


Figure 3.19 Mean diameter growth of seedlings over the two-year period: FCF 2



Seedling height was not different between treatments two years after planting ($F_{(2,4181)} = 2.87$, $P > 0.05$). With the exception of site CHH1 where seedlings treated with 15g Marshal suSCon® grew less than the other seedlings in the site, differences between treatments were either slight, or non-existent. As suggested, the lack of growth by seedlings treated with 15g Marshal suSCon® may have been due to random site effects at planting. Seedling height was significantly different between blocks ($F_{(6,4181)} = 4.11$, $P < 0.01$) and sites ($F_{(3,4181)} = 1456.93$, $P < 0.01$) after two years. Seedling diameter was not different between treatments after two years ($F_{(2,4181)} = 1.92$, $P > 0.05$). This indicates that treatment had no influence on the diameter growth of seedlings. Seedling diameter was significantly different between blocks ($F_{(6,4181)} = 2.51$, $P < 0.05$) and sites ($F_{(3,4181)} = 1526.74$, $P < 0.001$) at planting. Growth differences between blocks and sites were significant throughout the duration of the trial and indicate the influence of site effects on seedling growth. This was illustrated by the overall difference in growth of seedlings between the four trial sites. Differences in seedling growth within and between sites were expected and the trial was designed to account for this variation.

3.2.4 Discussion

Marshal suSCon® provided significant protection to *P. radiata* seedlings from *H. ater* attack. The treatment of seedlings by Marshal suSCon® reduced the incidence of tree death and damage by *H. ater*. The results are consistent with other studies evaluating the effectiveness of Marshal suSCon® to protect seedlings from bark beetle and weevil attack (Lemperière & Julien 1989, Mrlina *et al* 1994, Heritage *et al* 1997a). Information on the high numbers of seedlings attacked is important if sub-lethal attack by *H. ater* makes seedlings vulnerable to infection by invasive pathogens (e.g. fungi). It is possible that the significance of such infection may go undetected for many years, or may not be detected at all (Leather *et al* 1999). Further research was required to fully understand the implications of sub-lethal attack by *H. ater* on seedlings and forms the basis of Chapter Four.

The results show significant differences in the frequency of seedlings attacked between treatments. The frequency of sub-lethal seedling attack by bark beetles has been largely overlooked in many studies, which have instead focused on mortality. The high level of attack on untreated seedlings and the relatively low level of attack on treated seedlings

indicates that Marshal suSCon® had a repellent effect on *H. ater*. If the effect of sub-lethal attack by *H. ater* on seedlings were fully known, the possible benefits of protecting seedlings from any contact with *H. ater* could be properly appreciated.

In addition to reducing the frequency of *H. ater* attack, Marshal suSCon® applied to the seedlings at planting also reduced the severity of attack. On feeding, Marshal suSCon® is taken into the gut and kills the attacking beetle (Heritage *et al* 1997a). Seedling attack is therefore generally light as feeding activity is stopped soon after it is initiated. A reduction in the severity of seedling attack may enhance the benefits of using Marshal suSCon® if the susceptibility of a seedling to infection (e.g. by pathogens) increases with increasing severity of attack (Chapter Four).

This study shows that seedlings are capable of recovering from severe *H. ater* attack. No seedlings died from *H. ater* attack after one year. The decrease in severity of attack of seedlings in the second year after planting (Figure 3.11) compared with one year following planting (Figure 3.10) indicates that seedlings recover from severe attack. *H. ater* attacks seedlings below the soil surface during summer/autumn. Increased soil moisture over the winter months may aid seedling recovery. Damage observed two years after planting was likely to be due to attack during the previous year (which seedlings failed to fully recover from). In most cases seedlings had continued to grow despite this attack. When attack does occur, it is likely that extreme environmental events (i.e. drought, severe frosts) will compromise the health of severely attacked seedlings. While the majority of seedlings that were severely attacked in this study were able to recover, it is possible that at other times (i.e. during periods of drought) the chance of successful recovery may not be as high. On occasions where episodes of severe feeding on seedlings by *H. ater* are followed by extreme environmental events, the benefits of protecting seedlings with Marshal suSCon® may be more apparent.

Marshal suSCon® did not have a phytotoxic effect on the seedlings in this study. While Marshal suSCon® is reported to be phytotoxic to *Pinus patula* (Atkinson & Govender 1997), its application did not have a phytotoxic effect on *P. radiata* seedlings. Marshal suSCon® granules have been found to be phytotoxic to germinating seedlings (Heritage *et al* 1997a).

It was anticipated that seedlings might have benefited from the application of Marshal suSCon® as they would not have had to use resources to heal wounds. Instead energy could be used for plant growth. Örlander and Nilsson (1999) observed that seedlings protected from *Hylobius abietis* attack grew considerably faster than untreated seedlings. The reduction in growth was attributed to disturbances in the nutrient and water transport systems of the untreated seedlings (Örlander & Nilsson 1999). The use of resources to heal wounds would be an additional energy drain for attacked seedlings (Örlander & Nilsson 1999). As Leather *et al* (1999) have indicated, it is possible that seedlings that are moderately damaged will experience negative growth effects years after the damage was sustained. It is possible that the full benefits of Marshal suSCon® protection to seedlings in this trial will not be fully realised for some time.

In conclusion, Marshal suSCon® was an effective means of protecting *P. radiata* seedlings from mortality resulting from *H. ater* attack. Seedlings treated with Marshal suSCon® were attacked less frequently and with less severity compared to untreated seedlings. Marshal suSCon® did not have a phytotoxic effect on seedlings.

4. SECONDARY EFFECTS OF *H. ater* ATTACK ON *P. radiata* SEEDLINGS

4.1 Introduction

Scolytids are known worldwide as vectors of fungi. The interactions between aggressive bark beetles and fungi are well documented. These fungi play an important role in the tree killing by bark beetles (e.g. members of the genus *Dendroctonus*) (Birch 1978, Klepzig *et al* 1991, Raffa 1991, Paine *et al* 1997). Host responses are complicated when fungi associated with bark beetles are involved (Smalley *et al* 1993). Not all species of fungi may be beneficial to the beetle that carries them. Fungi benefit from the relationship with beetles by being dispersed. Benefits to the beetle are not clear (Harrington 1993b). Most authors support the notion that beetles and fungi are mutualistic symbionts (Harrington 1993b). Many species of bark beetles (including species of *Hylastes*) have specialised structures, or mycangia, which are typically simple pits found on the head, pronotum or elytral areas that carry fungal spores (Klepzig 1998, Harrington 1993b, Paine *et al* 1993, Solheim 1993). These mycangia most likely did not evolve to carry species of *Ophiostoma* and are often filled with yeasts and other fungi that are nutritionally beneficial to bark beetles. These species may compete with *Ophiostoma* species by inhibiting their development (Harrington 1993b). Spores may also be carried internally within the digestive tracts (Harrington 1993b, Malloch & Blackwell 1993, Solheim 1993).

Staining fungi are a significant economic concern to the *P. radiata* forest industry (Butcher 1967, 1968, Farrell *et al* 1997), due to the high susceptibility of *P. radiata* wood to staining (Wingfield & Gibbs 1991). Sapstain fungi mainly grow in wood in the ray parenchyma cells, within resin canals, tracheids and fibre cells, and penetrate simple and bordered pits (Ballard *et al* 1982, Farrell *et al* 1997). Parenchyma cells are the only living tissue in the xylem. Ray parenchyma cells store food as starch and protein (Zimmerman & Brown 1977). The invasion of wood by sapstain fungi is characterised by staining of the sapwood. This discoloration may be grey, black or brown reflecting the pigment of the fungal hyphae (Seifert 1993). This staining is due to pure melanin or melanin associated with carbohydrates and proteinaceous components, on small granules on the outer surface

of the fungus wall and in the medium surrounding the cells (Wheeler 1983, Zink & Fengel 1988, Brisson *et al* 1996, Eagen *et al* 1997). The pigment produced by the fungus stains the wood cells (Brisson *et al* 1996). At least three fungal groups cause sapstain in lumber: species of *Graphium*, *Ophiostoma* and *Leptographium*; black yeasts and dark moulds (Seifert 1993). While damage to the wood is suggested to be cosmetic, some species cause a reduction in the strength of wood (Seifert 1993). Toughness is the wood property most affected by sapstain, and may be reduced by 30% in severely stained pine (Seifert 1993). Some species also cause slight losses in specific gravity, surface hardness, and bending and crushing strength (Chapman & Scheffer 1940). Species of *Ophiostoma* may cause weight losses in conifer and angiosperm wood (Seifert 1993).

Saprophytic fungi and pathogenic and endophytic fungi cause sapstain in wood. Saprophytic fungi are thought to be of greater economic significance, as this group invades timber after the tree has been harvested (Seifert 1993). The staining effect only becomes evident when conditions are favourable for fungal growth. The most favourable conditions for growth are found in wood with high sapwood content in a warm humid climate (Kay *et al* 1997, Seifert 1993). Staining due to pathogenic and endophytic fungi is apparent when the tree is harvested and wood may be discarded prior to processing (Seifert 1993).

Sapstain fungi have been recorded from less aggressive bark beetles, in particular from members of the genus *Hylastes*. Species of *Leptographium* and *Graphium* have been found on *H. ater* in Britain, South Africa and Australia (Wingfield & Gibbs 1991, Dowding 1973). In New Zealand *Leptographium* (= *Verticicladiella*) has been isolated from *H. ater* (MacKenzie & Dick 1984). Species of *Hylastes* are known vectors of fungal root diseases in other parts of the world (Wingfield & Knox-Davies 1980, Witcosky & Hansen 1985, Witcosky *et al* 1986a). In these cases, *Hylastes* adults attack the roots of stressed or diseased adult trees and vector root disease fungi (Wingfield & Knox-Davies 1980, Witcosky & Hansen 1985, Jacobi 1992). Rudinsky and Zethner-Møller (1967) suggest feeding by species of *Hylastes* on seedlings may allow the fungus *Armilaria mellea* to enter through the feeding wound.

Mortality resulting from *H. ater* attack may not be high in many sites, yet significant levels of sub-lethal attack are common (Chapter Three). Sub-lethal attack does not appear to have any primary effect on seedlings (i.e. influencing growth). However, the process of

feeding by *H. ater*, or the creation of a wound resulting from feeding, may be detrimental to seedling health. This study provided an opportunity to investigate whether any relationship existed between sub-lethal feeding by *H. ater* and the subsequent invasion of seedlings by sapstain fungi and other fungal pathogens.

If *H. ater* is a vector for sapstain fungi in New Zealand and is capable of transmitting these fungi to seedlings during sub-lethal attacks, there could be significant implications for the New Zealand *P. radiata* industry. The role of *H. ater* and sapstain would need to be evaluated. Reducing the incidence of sapstain may be possible with control of *H. ater* if a relationship is identified. The pest status of *H. ater* may have to be re-assessed in New Zealand with the possibility of new fungal and disease introductions associated with bark beetles (Fox *et al* 1991, Dick & Bain 1996, Dick 1998).

The objectives of this study were to:

- Determine whether *H. ater* vectored sapstain fungi
- Determine whether live seedlings attacked by *H. ater* contained sapstain fungi
- Determine if sapstain fungi were absent from seedlings that were not attacked by *H. ater*
- Quantify the incidence of sapstain fungi from seedlings attacked by *H. ater*
- Determine whether the incidence of sapstain fungi isolated from seedlings was positively correlated with increasing severity of *H. ater* attack
- Identify to species level, the sapstain fungi isolated from seedlings and *H. ater* in all the above objectives

4.2 Methods

Eleven sites were chosen at random in second rotation *P. radiata* forests in the central North Island of New Zealand. These 11 sites were also included in the study assessing the extent of seedling attack by *H. ater*, the topic of Chapter Three. The sites were planted during the winter of 1998.

Seedlings were selected along a randomly located transect in each site. Every fifth seedling intercepted was destructively sampled, until a total of 15 seedlings had been

sampled in each site. Seedlings were removed from the ground and examined for evidence of attack by *H. ater*, as follows (refer to section 3.1.2 for a detailed description):

0= No evidence of attack

1= Mild attack

2= Moderate attack

3= Severe attack

Each seedling sampled was sealed in a carefully labelled plastic bag and stored in a freezer at -20°C until isolations of sapstain fungi were made from it.

Roberta Farrell and Arvina Ram, Department of Biological Sciences, University of Waikato, Hamilton made all isolations of sapstain fungi. The Waikato group, and Doug McNew and Thomas Harrington of Iowa State University identified all sapstain fungi isolated, using the methods described below.

Seedlings were surface sterilised by soaking them in 2% hypochlorite solution for one minute, and were rinsed twice using distilled water. Stem samples were then sliced into slithers using a sterile scalpel and placed onto selective media as follows:

Adult *H. ater* were collected from a variety of sites (unrelated to those sites where seedlings were sampled). Specimens collected were colonising freshly cut *P. radiata* logs and were collected from under the bark of *P. radiata* stumps. Individuals collected from different locations were stored separately.

All isolations and identifications of fungi from *H. ater* adults were done as follows: Adult *H. ater* were either placed directly onto the two media or were surface sterilised for one minute in 2% hypochlorite solution and rinsed twice in distilled water before being crushed and placed on both media.

Two selective media were used. The first, Media 4 consisted of yeast malt (YM) agar (0.2% yeast extract, 2.0% malt extract and 2.0% agar) with 200 micrograms per ml chloramphenicol and 100 micrograms per ml streptomycin. The second, Media 6 consisted

of YM agar (as above) and 200 micrograms per ml chloramphenicol, 100 micrograms per ml streptomycin and 400 micrograms per ml cycloheximide.

The plates were incubated at 25°C for up to 30 days. As fungi developed, cultures were transferred aseptically onto fresh plates. Cultures were identified on the basis of morphological features using classical microbial techniques as well as molecular DNA probing (Harrington *et al* 2001).

The relationship between the presence of sapstain fungi and seedling attack by *H. ater* was assessed using the non-parametric chi-square test, using the statistical package SAS (PROC FREQ, Version 6.12 for Windows, SAS Institute 1996). Pairwise comparisons were conducted using Holm's sequential Bonferroni method to determine the nature of the differences detected by chi-square.

Analysis of variance (ANOVA) was used to compare the relationship of the severity of *H. ater* attack and invasion by sapstain fungi, using the statistical package SAS (PROC GLM, Version 6.12 for Windows, SAS Institute 1996). Pairwise multiple comparisons were conducted using Duncan's multiple range test to determine the nature of the differences detected by ANOVA.

4.3 Results

Fungal isolations from seedlings

Six species of sapstain fungi were isolated from the live seedlings in this study (Table 4.1). These were *Ophiostoma huntii* (Robinson-Jeffery) de Hoog & R.J. Scheff, *Ophiostoma galeiformis* (Bakshi), *Ophiostoma piceae*, *Ophiostoma quercus* (Georgév.) Nannf., *Leptographium procerum* and *Leptographium truncatum*. Many other species of fungi were isolated from the seedlings, including *Penicillium* sp., *Trichoderma* sp., *Rhizopus* sp. and *Alternaria* sp. Other species of sapstain fungi may have been present, but could have either been rare or missed by the selection procedure.

O. huntii was the most common sapstain fungi isolated from 23 seedlings (Table 4.1). The isolation of more than one species of sapstain from seedlings was not uncommon. Three was the greatest number of sapstain species isolated from one seedling.

Table 4.1 The number of *P.radiata* seedlings from which sapstain fungi were isolated.

Sapstain species	Number of infected seedlings
<i>Ophiostoma huntii</i>	23
<i>Ophiostoma galeiformis</i>	12
<i>Ophiostoma piceae</i>	9
<i>Ophiostoma quercus</i>	3
<i>Leptographium procerum</i>	6
<i>Leptographium truncatum</i>	1

(total of 165 seedlings)

The majority (68.5%) of seedlings sampled in this study was attacked by *H. ater* (Table 4.2). Of these 23% were severely attacked, while 25.5% and 20% showed evidence of moderate and mild attack respectively (Table 4.2). Approximately one third (31.5%) of seedlings were not attacked by *H. ater*.

Table 4.2 The number of seedlings attacked by *H. ater*.

	Severity of attack				Total number of seedlings attacked
	No attack	Mild attack	Moderate attack	Severe attack	
Number of seedlings	52/165	33/165	42/165	38/165	113/165
Percentage of seedlings	31.5%	20%	25.5%	23%	68.5%

Sapstain fungi were isolated from 24.8% of seedlings in the study (Table 4.3). Half of the seedlings severely attacked by *H. ater* contained sapstain fungi (Table 4.3). Of those seedlings moderately attacked by *H. ater*, 28.6% contained sapstain fungi. Twelve percent

of seedlings attacked with mild severity and 11.5% of seedlings not attacked by *H. ater* contained sapstain fungi (Table 4.3).

Table 4.3 Number of seedlings infected by sapstain fungi.

	Severity of attack				Total number seedlings attacked and infected by sapstain fungi
	No attack	Mild attack	Moderate attack	Severe attack	
Number of seedlings infected by sapstain fungi	6/52	4/33	12/42	19/38	41/165
Percentage of seedlings	11.5%	12%	28.6%	50%	24.8%

The presence of sapstain fungi on seedlings attacked by *H. ater* was greater than those that were not attacked ($X^2_{(1)}= 7.203, P< 0.01$). The presence of sapstain fungi infecting seedlings was influenced by the severity of *H. ater* attack ($X^2_{(3)}= 20.980, P< 0.001$). As the severity of attack increased, so did the likelihood of infection (Table 4.4). The presence of sapstain fungi infecting seedlings was different between sites ($X^2_{(10)}= 34.337, P< 0.001$).

Table 4.4 Results for the pairwise comparisons using Holm’s sequential Bonferroni method: Mean frequency of seedlings infected by sapstain fungi.

Severity of attack by <i>H. ater</i>	Mean number of seedlings infected (%)	Number of seedlings sampled
Severe	50.0 _a	38
Moderate	28.6 _b	42
Mild	12.0 _c	33
No attack	11.5 _c	52

Means with the same letter are not significantly different (alpha= 0.05).

The number of species of sapstain fungi isolated from seedlings did not differ with the presence of attack ($X^2_{(3)} = 7.550, P > 0.05$). The number of species of sapstain fungi isolated from seedlings did differ with severity of attack ($F_{(3,151)} = 8.4, P < 0.001$). Those seedlings severely and moderately attacked by *H. ater* were most likely to be infected by more species of sapstain fungi (Table 4.5). Seedlings not attacked by *H. ater* and those attacked with mild severity were least likely to be infected by more than one sapstain species (Table 4.5). The mean number of sapstain species isolated from seedlings differed between sites ($F_{(10,151)} = 2.85, P < 0.01$).

Table 4.5 Results for the pairwise comparisons using Duncan’s multiple range test: Mean number of sapstain fungi isolated from seedlings.

Severity of attack by <i>H. ater</i>	Mean number of sapstain species per seedling	Number of seedlings sampled
Severe	0.66 _a	38
Moderate	0.43 _a	42
Mild	0.13 _b	52
No attack	0.12 _b	33

Means with the same letter are not significantly different (alpha= 0.05).

Fungal isolations from *H. ater*

Ophiostoma ips (Rumb.) Nannf. was isolated from beetles found invading fresh billets in the field. *O. ips* was isolated from the surface of unsterilised adults and from the gut of surface sterilised adults. Other fungal species, including *Penicillium* sp., *Trichoderma* sp., *Rhizopus* sp. and *Alternaria* sp. were isolated from the surface and gut of the beetles. It is possible that other fungal species were also present.

4.4 Discussion

H. ater is a vector of sapstain fungi in second rotation *P. radiata* forests. In addition to *O. ips* being isolated in this study, other studies have isolated sapstain fungi from *H. ater* (Wingfield & Marasas 1980, Wingfield *et al* 1988a, Wingfield & Gibbs 1991). *O. ips* is commonly associated with bark beetles (Harrington 1988, Malloch & Blackwell 1993, Harrington 1993a,b) and will be discussed further. Other sapstain fungi isolated from *H. ater* in New Zealand include *Leptographium truncatum* (Wingfield & Gibbs 1991). *L. truncatum* was first described from New Zealand and South Africa. This species was found to be associated with the roots of dying trees infested with *Hylastes angustatus* in South Africa (Wingfield & Marasas 1983). In New Zealand *L. truncatum* is found associated with *H. ater* and *H. ligniperda* (Wingfield *et al* 1988a). When inoculated into pine stumps, *L. truncatum* causes distinct lesions (Wingfield & Marasas 1983). *L. lundbergii* has been reported to be associated with *H. ater* in New Zealand by Wingfield *et al* (1988a) and with *H. ater* in Britain (Dowding 1973). Wingfield and Gibbs (1991) and Strydom *et al* (1997) suggested the taxonomic status of *L. truncatum* should be reconsidered, and they argued that the species be reduced to synonymy with *Leptographium lundbergii*. Hausner *et al* (2000) report that the species concept of *L. lundbergii* might require reassessment and that confusion concerning this species is in part due to the lack of a type. However, Hausner *et al* (2000) suggest that Strydom *et al* (1997) did not include the Norwegian isolate of *L. lundbergii* NRFI 69-148 in their analysis (suggesting it was *O. huntii* instead) even though data seem to suggest this stain holds an intermediate position between *L. lundbergii* and *O. huntii*. Until the confusion regarding the taxonomic status of *L. lundbergii* is resolved it seems best to use *L. lundbergii*.

The presence of *Leptographium procerum* in New Zealand is likely to be due to its introduction with either *H. ater* or *H. ligniperda* (Wingfield & Gibbs 1991). *L. procerum* causes procerum root disease and has been associated with a number of bark beetles and weevils which attack conifer roots (i.e. *Hylobius* sp. (Klepzig *et al* 1991)) and will be discussed further. *O. huntii* is also likely to have been introduced into New Zealand with *H. ater* (Jacobs *et al* 1998).

Other species of sapstain are reported to be associated with *H. ater* in other countries (e.g. *O. minus* (Hedgecock) H. & P. Sydow, Gibbs 1993). *H. ater* is likely to be associated with

more species than were isolated in this study. In this study relatively few *H. ater* adults were investigated as vectors of sapstain fungi. The presence of other fungi (e.g. *Penicillium* sp., *Trichoderma* sp., *Rhizopus* sp., *Alternaria* sp.) indicate the potential for beetles to vector a wide range of fungal species. As *H. ater* breeds in stumps following harvesting activities, fungal spores found on *H. ater* are most likely to be associated with fungal species found in stumps (Harrington 1993b). A more comprehensive sampling programme would be expected to isolate a greater number of sapstain species from *H. ater*. The results show that there was a strong relationship between *H. ater* attack and the invasion of seedlings by sapstain fungi. This is the first time such an association has been demonstrated in New Zealand. The relationship between *H. ater* attack and the presence of sapstain fungi is not unequivocal evidence that fungal spores are being transmitted directly from beetles to seedlings during attacks. However, the relationship between *Hylastes* species and sapstain fungi overseas (Wingfield & Knox-Davies 1980, Witcosky & Hansen 1985, Jacobi 1992) indicates that this is a likely possibility.

Black-stain root disease is caused by *Leptographium wagnerii* (Kendrick) Wingfield. *L. wagnerii* colonises the roots, root collar and lower stems of Douglas-fir (*Pseudotsuga menziesii*) and species of *Pinus* (Smith 1967, Harrington & Cobb 1983, Harrington 1993b). *L. wagnerii* may kill mature trees, and causes a black ring-like stain in infected sapwood (Witcosky & Hansen 1985). Most other staining fungi cause a wedge-like stain (Witcosky & Hansen 1985). The disease may be transmitted from diseased trees to healthy trees in three ways (Witcosky & Hansen 1985). Firstly, by contact between major roots. Secondly, fungal hyphae may grow short distances through soil to roots. Finally, the disease may be transmitted to healthy hosts by insect vectors.

Hylastes nigrinus has been demonstrated to vector *L. wagnerii* to Douglas-fir (Harrington *et al* 1985, Witcosky *et al* 1986a,b, Harrington & Wingfield 1998). *Pissodes fasciatus* Le Conte and *Steremnius carinatus* (Boheman) also vector *L. wagnerii* (Witcosky *et al* 1986b, Harrington 1993b). *P. fasciatus* does not feed on living trees and *S. carinatus* does not fly, factors which limit their effectiveness as vectors (Harrington 1993b). Harrington *et al* (1985) suggest deep wounds by *H. nigrinus* are probably required for the infection of Douglas-fir by *L. wagnerii*. Activities such as pre-commercial thinning appear to increase the abundance of insect vectors of *L. wagnerii*, and consequently the impacts of the disease (Witcosky *et al* 1986a, Harrington & Wingfield 1998). *Hylastes macer* Le Conte

has been associated with *L. wagnerii* root disease in ponderosa pine (Goheen & Cobb 1978, Goheen & Hansen 1993).

Procerum root disease is caused by the fungi *Leptographium procerum* and infects many *Pinus* species (Lewis & Alexander 1986, Nevill & Alexander 1992). *L. procerum* is responsible for significant losses in Christmas tree (*Pinus strobus* L.) plantations in the eastern United States (Lewis & Alexander 1986, Nevill & Alexander 1992). *Hylastes* species and a number of weevil species have been found to carry *L. procerum*. Insect vectors are probably the primary modes of dispersal and inoculation (Lewis & Alexander 1986). *Hylobius pales* (Herbst) and *Pissodes nemorensis* Germar vector *L. procerum* (and *O. piceae*) to eastern white pine seedlings during sub-lethal attack (Nevill & Alexander 1992). Procerum root disease is an insect fungal complex. *L. procerum* and *L. terebrantis* Barras & Perry are vectored to the roots and lower trunk of mature red pines by *Hylastes porculus* Er. and *Dendroctonus valens* Le Conte (Klepzig *et al* 1996a). This complex is not lethal and infected trees only die when colonised by *Ips pini* (Say) vectoring *O. ips* (Klepzig *et al* 1996a).

In South Africa *Hylastes angustatus* carries the fungus *Leptographium serpens* (Wingfield *et al* 1988b). *L. serpens* causes well-developed lesions in *Pinus pinaster* inoculated roots. Unlike *L. wagnerii*, *L. serpens* does not kill seedlings in pots (Wingfield *et al* 1988b). The role of the species in root diseases in South Africa has been questioned, but it is an important component of *P. pinaster* root disease (Wingfield *et al* 1988b).

An alternative pathway for the invasion of seedlings by sapstain fungi from the soil may be via wounds, as *H. ater* attacks seedlings below the ground. Fungi may be transmitted to wounded seedlings from the soil following attack. A survey of the sapstain species in New Zealand (including samples from the forest floor) by Farrell *et al* (1997) demonstrated that Ophiostomataceae were isolated from 28% of the pine plantations sampled. While the mode of infection is not currently known, the association between *H. ater* attack and the invasion of seedlings by sapstain fungi is very significant. This new information is of concern and indicates that the pest status of *H. ater* in *P. radiata* forests may need to be re-evaluated.

The sapstain species isolated from *P. radiata* seedlings in this study are from the Ophiostomataceae (Malloch & Blackwell 1993, Farrell *et al* 1997). *Ophiostoma* is the largest genus of this group. Most *Ophiostoma* species inhabit living or recently dead wood and are commonly found in the galleries of bark beetles (Malloch & Blackwell 1993). Two *Ophiostoma* species, *O. huntii* and *O. galeiformis*, were the most frequently isolated from *P. radiata* seedlings attacked by *H. ater* in this study. *O. huntii* is a blue-stain fungus and was described from Canada by Robinson-Jeffrey and Grinchenko (1964) as being similar to *O. europioides* (E.F. Wright & Cain) H. Solheim. More recently, *O. huntii* has been isolated from many parts of the world (Jacobs *et al* 1998). *O. huntii* has been associated with several species of bark beetles, including *H. ater* (Harrington 1988, Jacobs *et al* 1998). *O. huntii* has been isolated from *Hylastes porculus* and may be an important species in red pine decline, an insect fungal complex that kills red pine (*Pinus resinosa* Ait.) (Klepzig *et al* 1991). *Leptographium procerum* and *L. terebrantis* and a number of other bark beetles are also associated with this complex (Klepzig *et al* 1991).

O. galeiformis is a European species which has been found with *Hylurgops palliatus* on larch in Scotland (Bakshi 1951a,b) and *Hylastes cunicularius* in Sweden (Mathiesen-Käärrik 1960). Mathiesen-Käärrik (1960) describes *O. galeiformis* as a secondary staining fungus. These utilise fewer carbon compounds than primary staining species of fungi (Mathiesen-Käärrik 1960). *O. galeiformis* was likely introduced into New Zealand with *H. ater* (T.C. Harrington personal communication).

The remaining sapstain species isolated from seedlings in this study are commonly found in New Zealand pine plantations (Farrell *et al* 1997, Thwaites 1999). *Ophiostoma piceae* is described as the most common sapstain species in Canada (Seifert 1993) and is a weak bark parasite of worldwide distribution (Pipe *et al* 1995). Blue to gray-black staining caused by fungal hyphae appears in wood a few days following inoculation (Breuil *et al* 1988), although Seifert (1993) suggests there appears to be some uncertainty as to the severity of the staining. *O. piceae* invades freshly cut ends of wood by aerial mechanisms and development is restricted to the sapwood surfaces close to the ends (Griffen 1968). *O. piceae* is regarded as one of the most aggressive sapwood staining fungi in Canadian lumber mills (Gao & Breuil 1995). Although *O. piceae* causes minor weight loss in timber, it does not effect the structural properties of wood (Abraham *et al* 1993).

O. quercus was first isolated from declining oaks in Europe and was regarded as a synonym of *O. piceae* (Brasier & Kirk 1993, Pipe *et al* 1995). In the Northern Hemisphere, two distinct populations are found from different hosts, *O. piceae* C (OPC) from conifers, and *O. piceae* H (OPH), associated with hardwoods (Brasier & Kirk 1993, Brasier & Webber 1991, Halmschlager *et al* 1994, Pipe *et al* 1995). Halmschlager *et al* (1994) and Pipe *et al* (1995) recommend that *O. quercus* be used rather than *O. piceae* H (OPH) to name the species. The two species exhibit different temperature growth responses (Brasier & Stephens 1993). In New Zealand *O. quercus* is isolated as frequently as *O. piceae* from felled *P. radiata* which makes the New Zealand situation atypical compared with that in the Northern Hemisphere (Farrell *et al* 1997). In New Zealand, *O. quercus* was also isolated from *Cupressus macrocarpa* (R.L. Farrell, personal communication).

Leptographium procerum and *L. truncatum* cause staining in timber and have been isolated from roots of diseased pines in New Zealand (Wingfield & Marasas 1983, Wingfield & Gibbs 1991). Most species of *Leptographium* are associated with insects that infest trees and are commonly found in insect galleries in wood (Harrington 1988, Harrington 1993b). Many species have been shown to be pathogenic to conifers and are thought to play an important role in the killing of live trees by bark beetles (Harrington 1993b). *L. procerum* is consistently isolated from declining red pine roots and is thought to be an important species contributing to this complex (Lewis & Alexander 1986, Klepzig *et al* 1991, Klepzig *et al* 1996b). *L. procerum* causes lesions in phloem and staining in sapwood, and is mildly pathogenic (Harrington & Cobb 1983, Harrington 1988, Klepzig *et al* 1991). It has been suggested that *L. procerum* is not as virulent as *Leptographium terebrantis*, which is found with *L. procerum* in the red pine decline complex (Klepzig *et al* 1996b).

One of the most common species of the Ophiostomatoid group, *O. ips*, was not isolated from seedlings despite being isolated from *H. ater* in this study. *O. ips* is regarded as one of the most common causes of staining in pine wood (Hunt 1956, Griffen 1968, Smalley *et al* 1993, Farrell *et al* 1997). This species has been recorded in many parts of the world including the United States (Rumbold 1931, Hunt 1956), Sweden (Mathiesen-Käärik 1960), South Africa (Wingfield & Marasas 1980), Japan and Poland (Benade *et al* 1995). Seifert (1993) describes *O. ips* and *O. piliferum* as the most common species found with conifers in the southern United States. This species is almost always associated with bark

beetles (Griffen 1968, Malloch & Blackwell 1993, Smalley *et al* 1993, Benade *et al* 1995). *O. ips* ascospores are dispersed in conifer resin but not water. It has been suggested that ascospores adhere to insect vectors and are then dispersed when they come in contact with resin (Malloch & Blackwell 1993). *O. ips* is less pathogenic than other *Ophiostoma* species, which may be weak parasites or saprophytes (Smalley *et al* 1993). *O. ips* may invade only highly susceptible hosts (e.g. during time of stress) and hosts may be resistant during conditions of favourable growth (Smalley *et al* 1993). *O. ips* vectored by *Ips pini* is suggested to be the cause of death in red pines that have been previously colonised and placed under stress by root and lower stem infesting beetles with the fungi *Leptographium terebrantis* and *L. procerum* (Klepzig *et al* 1991, Klepzig *et al* 1996a). *O. ips* causes a dark stain in wood (Seifert 1993).

While *O. ips* was not isolated from seedlings in this study, this may be a reflection of the distribution of sapstain species in those sites sampled. Alternatively, it is possible that seedlings were growing vigorously when attacked by *H. ater* and were able to resist invasion by *O. ips* (Smalley *et al* 1993). The anomaly surrounding *O. ips* isolated from *H. ater*, but not sub-lethally attacked seedlings in this study, is an indication that further investigation needs to be undertaken in this area.

The results clearly demonstrate an association between sapstain fungi and *H. ater* seedling attack. While the six species of sapstain fungi isolated from seedlings were not isolated from *H. ater* in this study, four have been isolated from *H. ater* in New Zealand in the past (Wingfield *et al* 1988a, Wingfield & Gibbs 1991, Jacobs *et al* 1998). Leather *et al* (1999) suggest attack by *Hylobius abietis* may result in the infection of pine seedlings by *Leptographium procerum*. Leather *et al* (1999) suggest if seedlings are infected during attack, mortality from otherwise minor feeding may be increased.

Rules proving the transmission of plant pathogens by Leach (1940), are similar to Koch's postulate (Whetzel 1918). In order to demonstrate that insects vector a disease to a host, four rules must be satisfied (Leach 1940). Firstly, insects must be associated with diseased hosts. Secondly, they must visit healthy hosts under conditions suitable for the transmission of the pathogen. Thirdly, insects must carry inoculum in the field. Finally, it must be demonstrated that insects can successfully transmit the pathogen to the host under laboratory conditions.

While an association has been suggested between *Hylobius abietis* and *Leptographium procerum* (Leather *et al* 1999), not all of the above conditions have been satisfied. Witcosky and Hansen (1985) and Witcosky *et al* (1986a,b) have satisfied all four conditions to confirm that *Hylastes nigrinus*, *Pissodes fasciatus* and *Steremnius carinatus* serve as vectors of *Leptographium wagnerii* to Douglas-fir.

With regard to the role of *H. ater* as a vector of sapstain fungi to *P. radiata* seedlings in New Zealand, some of the above conditions have been satisfied. Conditions one and two of the above were satisfied during this project (Chapter Three). The third condition, that *H. ater* must carry an inoculum of the sapstain fungi in the field, has been satisfied for *O. ips* in this study and for other sapstain species from other studies (Wingfield *et al* 1988a, Wingfield & Gibbs 1991, Jacobs *et al* 1998). The final condition was not studied, and it is unknown if it would be satisfied. Therefore, it is not possible to state unequivocally that *H. ater* vectors sapstain fungi to *P. radiata* seedlings. However, the results from this study do indicate that with further investigation, it should be possible to confirm condition three and to test and satisfy condition four.

Sphaeropsis sapinea (Fries) Dyko & Sutton (= *Diplodia pinea*) was not isolated from seedlings in this study. *S. sapinea* belongs to the Fungi Imperfecti group and exists as an endophyte in *P. radiata* at pruned sites and beneath the bark of healthy trees (Birch 1936, Chou 1984, Farrell *et al* 1997, Harrington & Wingfield 1998). *S. sapinea* is regarded as the most important cause of sapstain in wood, and invades trees after harvesting (Farrell *et al* 1997). Other fungal species may colonise when competition from *S. sapinea* is minimal (Butcher 1967). *S. sapinea* is able to invade undamaged green shoots of *P. radiata* (Chou 1976), resulting in shoot dieback and occasional tree death. This may be a serious problem in localised areas only (Chou 1984). The absence of *S. sapinea* in this study is of interest to the forest industry. However, further research is required before invasion by *S. sapinea* of seedlings attacked by *H. ater* can be discounted.

This study was intended to be a preliminary investigation of potential relationships between sapstain fungi and seedling attacks by *H. ater*. Farrell *et al* (1997) suggest that Ophiostomataceae are found in approximately one quarter of pine plantations. It is possible that many species present in the forest were not present in the sites sampled in this study. It is also possible that *P. radiata* seedlings are able to resist invasion by some

sapstain fungi (Smalley *et al* 1993, Solheim 1993). Further research is required to investigate the relationship between seedling attack and infection by different species of sapstain fungi.

The relationship between the severity of *H. ater* attack and the subsequent colonisation of seedlings by sapstain fungi is of interest to the forest industry. The results show that those seedlings that were severely attacked by *H. ater* were at the greatest risk of being infected by sapstain fungi. While this was a preliminary study, half of severely attacked seedlings were colonised by sapstain fungi. There are potentially enormous numbers of seedlings in the forests infected by sapstain fungi. The results of the destructive sampling programme described in Chapter Three indicate that severe attack by *H. ater* is common in many plantings, especially those sites harvested during the end of summer. If the effects of sapstain fungi on *P. radiata* seedlings were fully known, it may be possible to estimate the impact of this phenomenon on the forest industry. But until further research is initiated to investigate this relationship, the potential consequences of this association can only be implied. However, greater caution should be given to planting high-risk areas.

Commonly, attack by *H. ater* is regarded as a cause of mortality in seedlings. The seedlings in this study survived attack by *H. ater*. The sapstain fungi associated with seedlings were isolated from beneath the bark following surface sterilisation. This indicates that sapstain fungi may persist in seedlings for some time (Nevill & Alexander 1992). A current survey of sapstain in New Zealand has found that sapstain fungi may be isolated from apparently healthy mature trees (Farrell *et al* 1997). It is not known whether the presence of sapstain in mature trees was the result of *H. ater* attack following establishment, or by other means.

Sapstain fungi were isolated from seedlings that had not been attacked by *H. ater*. The frequency of infection was not significant in comparison with attacked seedlings (particularly those severely attacked). The pathways of invasion are not known for these seedlings, but may be due to sapstain invading the seedling during planting or pre-planting treatments.

The incidence of sapstain infected seedlings varied between sites. *H. ater* related mortality and attack varies considerably within the forest estate (refer to Chapter Three). Some sites

are characterised by high *H. ater* attack. The presence of *H. ater* is patchy within the forest and seedling attack may be dependent on a variety of factors (refer to Chapter Three). While a survey of New Zealand sapstain fungi (Farrell *et al* 1997) indicates that the species of sapstain isolated from seedlings in this study are found throughout *P. radiata* forests, it is possible that fungal distribution may be uneven at smaller scales (i.e. between compartments). Seedlings in some sites may be severely attacked by *H. ater* and infected by proportionately low levels of sapstain fungi, and vice versa.

This study was the first investigation into *P. radiata* seedling attack by *H. ater* in second rotation forests in New Zealand and invasion by sapstain fungi. The potential for *H. ater* attack to cause seedlings to be infected by sapstain fungi is of great concern. However, further research needs to be undertaken to understand this relationship, and the consequences of it to the forest industry. The association between *H. ater* and sapstain fungi may have implications for New Zealand quarantine operations. *H. ater* is currently regarded as a non-risk, non-quarantine organism in New Zealand (MAF 2000). However if any *H. ater* individuals introduced into New Zealand are carrying a new suite of fungi, then by definition they may be classified as new organisms in terms of their pest potential.

The results indicate that the pest status of *H. ater* in New Zealand should be reconsidered. As *H. ater* has been suggested as the mechanism by which a number of species of fungi have been introduced into New Zealand (Wingfield & Gibbs 1991, Jacobs *et al* 1998), it is possible that future introductions may establish new fungal species (or other organisms). If other fungal pathogens are introduced into New Zealand, there is potential for *H. ater* to vector these in forests. The potential damage to the forest industry may be very serious if disease species such as *Leptographium wagnerii* were to become established in New Zealand. Other pathogens, such as pine pitch canker (*Fusarium subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun & Marasas f. sp. *pini*), pose a significant threat to forestry in New Zealand (Dick & Bain 1996, Dick 1998). *F. subglutinans* was first reported on *Pinus virginiana* Mill. (Hepting & Roth 1946). More recently pitch canker has been reported from California (McCain *et al* 1987), South Africa, Mexico and Japan (Viljoen *et al* 1994, Viljoen *et al* 1997, Harrington & Wingfield 1998). Pitch canker is pathogenic to *P. radiata* (Storer *et al* 1995), the most susceptible of the *Pinus* species (Dick & Bain 1996, Dick 1998). *F. subglutinans* is vectored by bark beetles in California

(Schmidt & Underhill 1974, Fox *et al* 1991, Storer *et al* 1995, Harrington & Wingfield 1998) and may be vectored by *H. ater* (Dick & Bain 1996, Dick 1998).

The areas of research listed below need to be initiated to further develop our understanding of the relationship between *H. ater* seedling attack and invasion by sapstain fungi. In addition, the role of *H. ater* in the movement of fungi in the forest system should be addressed. Only when these issues are addressed may the full implications of seedling infection by sapstain fungi be fully quantified. Any future research should:

- Investigate the effect of sapstain fungi on seedlings following sub-lethal attack
- Quantifying how long seedlings remain infected by sapstain fungi following *H. ater* attack by establishing some long term monitoring studies on the fate of seedlings attacked by *H. ater*
- Determine whether sapstain fungi continue to spread throughout seedlings following invasion, or are contained by subsequent tree growth
- Establishing the role of *H. ater* in vectoring fungi e.g. Are sapstain fungi directly vectored to seedlings by *H. ater* during attack, or do sapstain fungi invade seedling wounds from soil following *H. ater* attack?
- Investigating whether different seedling types (i.e. cuttings vs. seedlings, different genetic material) show different levels of response to invasion by sapstain fungi
- Further surveying of the sapstain fungi vectored by *H. ater* is required to understand why certain species are not associated with *H. ater*.

5. THE RESISTANCE OF *P. radiata* SEEDLINGS TO *H. ater* ATTACK

5.1 Introduction

The resistance of live tree hosts to bark beetle attack may be classified into two categories: primary or preformed resistance and induced resistance (Berryman 1972, Schroeder 1990, Lieutier 1993, Nebeker *et al* 1993, Lorio 1994, Paine *et al* 1997). Preformed resistance is the defence that exists in plants independently of attack by an organism. This first defence mechanism is the resin canal system (Berryman 1972). While this system is present in most of the Abietineae, it is absent from the genera *Abies*, *Tsuga*, *Cedrus*, *Pseudolarix* (Berryman 1972). Members of the genus *Pinus* have the most highly developed system of vertical and horizontal resin ducts (Nebeker *et al* 1993). Bark beetles that attack this system sever resin ducts and are overwhelmed by resin flow. This may prevent bark beetle establishment (Berryman 1972, Christiansen *et al* 1987, Raffa 1991, Lieutier 1993, Paine *et al* 1997). The resin consists of monoterpenes, sesquiterpenes and resin acids which flush the wound clean then seal the tissue with resin crystallisation (Christiansen *et al* 1987, Paine *et al* 1997, Smalley *et al* 1993). Attacking beetles can be forced out of their galleries. Sealing off the attack can prevent the escape of pheromones (Raffa & Berryman 1983, Raffa 1991, Paine *et al* 1997).

One factor limiting the effectiveness of the tree's passive reaction is duration of resin flow. Substantial amounts of resin may be lost from the attacking beetles' entry. Resin flow is unlikely to prevent the establishment of fungal invasion in all tissues (Berryman 1972, Nebeker *et al* 1993). The extent of the host response is influenced by the season, host genetics and age, and may be reduced by stress (Smalley *et al* 1993). Primary resistance places significant energy demands on host trees (Paine *et al* 1997). The two factors of this system that confer resistance to beetle attack are, firstly, the chemical composition of the resin and, secondly, the physical properties of the resin pressure, flow and crystallisation (Paine *et al* 1997). While some resin components have anti-bacterial and fungal qualities, other components of resin may stimulate fungal growth (Paine *et al* 1997).

The induced or hypersensitive reaction is a tree's second line of defence following invasion or infection of the inner bark tissues, and is not well understood (Berryman 1972, Christiansen *et al* 1987, Lieutier 1993, Nebeker *et al* 1993, Klepzig *et al* 1996a, Paine *et al* 1997). This defence is an active metabolic process where terpenes, polyphenols and other compounds are released (Paine *et al* 1997). This reaction may be initiated by fungal infection (Lieutier 1993) rather than mechanical damage, and can seal both insects and fungi in a lesion of dead resin-impregnated tissue (Berryman 1972, Nebeker *et al* 1993, Klepzig *et al* 1996a). In some hosts, the hypersensitive reaction may be the most important defence mechanism. There are two main advantages of surrounding only the infected area. Carbohydrates are conserved, and the amount of cambium that must be replaced is minimised (Christiansen *et al* 1987, Schroeder 1990, Lieutier 1993).

The reaction may effect the insect in two ways (Berryman 1972, Nebeker *et al* 1993, Paine *et al* 1997). Firstly, the metabolites flow into the beetles gallery under pressure causing the beetle to be repelled or killed. Secondly, the resin soaked tissues are unsuitable for the survival of the eggs and larvae of bark beetles. Beetles that survive resinosis may experience detrimental effects to their reproductive systems (Lorio 1994, Paine *et al* 1997). In addition, the resin contains the spread of pathogenic fungi to a discrete area by forming fungal toxic or fungistatic compounds in advance of the fungus (Berryman 1972, Paine *et al* 1997). Those trees that can successfully resist colonisation by bark beetles and associated organisms produce an induced response (Paine *et al* 1997). This induced response is non-specific. However, infection by different fungi results in different intensities of response. Trees respond mostly to pathogenic fungi, in part caused by the rate of growth of different fungi (Paine *et al* 1997). The induced defence system may be exhausted by extensive and prolonged beetle attack, with the host tree being overcome once the threshold of attack has been reached (Berryman & Ashraf 1970, Raffa & Berryman 1983, Raffa 1991, Lorio 1994, Paine *et al* 1997). The attack threshold is a function of host vigour and is directly influenced by stress (Raffa & Berryman 1983, Christiansen *et al* 1987, Lorio 1994, Paine *et al* 1997). The threshold of attack is determined by resin reserves at the time of attack, and the capacity of the tree to mobilise defensive chemicals (Christiansen *et al* 1987). These depend on the carbohydrate content of a host (Christiansen *et al* 1987). Therefore, defence is directly related to the carbon balance of host trees. Any factor that influences the photosynthetic capability of a host may weaken its resistance.

Different species may allocate different resources to the two defence systems (Raffa & Berryman 1983, Raffa 1991, Paine *et al* 1997). Both host resistance mechanisms may be overcome by bark beetles. Mass colonisation may drain resources (Raffa & Berryman 1983, Raffa 1991, Paine *et al* 1997) and is influenced by a number of related factors. Low oleoresin pressure has been associated with host susceptibility to bark beetles (Vité 1961, Vité & Wood 1961, Rudinsky 1962, Wood 1962, Mason 1971, Schroeder 1990, Paine *et al* 1997), although Lorio (1994) suggests that measures of resin flow may not be a satisfactory method of estimating resistance. Trees of low vigour may be attacked more often, or may be overwhelmed by attacking beetles more easily than high-vigour trees (Mason 1971, Hodges *et al* 1979, Larsson *et al* 1983, Mitchell *et al* 1983, Matson *et al* 1987, Schroeder 1987).

While the effectiveness of a resin system is genetically controlled, it is also influenced by environmental factors (Mason 1971, Hodges *et al* 1979, Raffa & Berryman 1983, Wainhouse & Ashburner 1996, Lieutier *et al* 1997a,b Sahota *et al* 1994), such as season, drought and wind (Vité 1961, Lorio 1968, Kalkstein 1981, Matson *et al* 1987, Lownsbery 1988, Raffa 1991). Environmental factors also influence host condition. For example, root disease, physical injury and lightning strikes all effect resin composition and flow (Vité 1961, Cobb *et al* 1968, Christiansen *et al* 1987, Lownsbery 1988, Raffa 1991, Nebeker *et al* 1993, Paine *et al* 1997).

Improving growing conditions by thinning, site selection and other management practices can enhance characteristics of host defence systems (Mason 1971, Larsson *et al* 1983, Mitchell *et al* 1983, Matson *et al* 1987, Lownsbery 1988, Paine *et al* 1997). Host resistance is directly related to the availability of energy reserves that may be utilised to withstand attack. Any factor, environmental or otherwise, that may reduce the size of a host tree's canopy or its photosynthetic efficiency can effect host resistance (Berryman 1972, Christiansen *et al* 1987, Raffa 1991, Paine *et al* 1997).

Both the preformed and induced defence processes are affected by host energy balance. Trees may respond less to attacks during periods when carbohydrates are moved to growth processes (Lorio & Sommers 1986, Tuomi 1992). Hermes and Mattson (1992) and Tuomi (1992) suggest that host defence is a function of secondary metabolites. Three principles appear crucial to defence mechanisms (Hermes & Mattson 1992). Firstly, genetic

correlation between growth and defence is negative. Secondly, photosynthesis is not as sensitive as growth to environmental stress. Finally, plants that are dominated by growth have more plastic defences than differentiation dominated plants. During periods when moisture limits growth, trees respond more rapidly to bark beetle fungal associates (Lorio & Sommers 1986, Paine *et al* 1997). Both resistance mechanisms are connected to the host trees source-sink relations, with the allocation of resources to resistance mechanisms and tree growth being critical to changing resistance thresholds, and beetles host selection and colonisation behaviour (Lorio & Sommers 1986, Paine *et al* 1997).

While some beetles may be resistant to host resins (Raffa 1991), other species may tunnel into the host in a way that minimises flow from the severed ducts (Berryman 1972, Raffa 1991). The most effective mode of overcoming host defences is by beetles tunnelling horizontally across the grain. This means fewer beetles may be needed to disrupt the flow of water and nutrients to the tree (Raffa 1991). In addition, fungi may be inoculated by beetles boring around the circumference of a host, further disrupting the translocation of host fluids (Berryman 1972, Nebeker *et al* 1993). Those beetles that attack trees with good resin system defences usually bore tunnels which do not deeply score sapwood, and may frequently orientate their galleries vertically and upwards from the point of attack. By boring a short tunnel at an angle before proceeding vertically, the major vertical and anastomosing horizontal ducts may be drained by gravity (Berryman 1972).

Environmental factors are thought to play an important role in the host resistance of *P. radiata* seedlings to attack by *H. ater* (Zondag 1958, Zondag 1968). Environmental factors that influence seedling resistance to attack by *H. ater* may be any factor that compromises the vigour or health of seedlings (Leather *et al* 1999). It might be possible to identify the extent to which environmental factors influence the resistance potential of seedlings (Leather *et al* 1999). While little restorative action would be taken in such situations, it might be feasible to implement management or control strategies in these sites.

It is difficult to evaluate the environmental factors that influence resistance (Leather *et al* 1999). Other factors that affect seedling resistance attack may prove more useful. Investigating genetic variability may result in developing alternatives to manage *H. ater* attack. Some seedling types are likely to have genetic traits that make them more resistant to *H. ater* attack. Resistant seedlings may be planted to minimise the effects of *H. ater* in

high-risk sites. Tree resistance has been regarded as the ideal means with which to control insect pests (Wiseman 1994), yet historically this method has been under-utilised (Callahan 1964, Smith 1964, Hanover 1975, Hodges *et al* 1979). The resistance qualities of pines that are genetically controlled include viscosity, total flow and the rate of resin crystallisation (Hodges *et al* 1979).

Seedlings are high quality food and susceptibility to attack varies both within and between species (Alfaro & Ying 1990, Sahota *et al* 1994, Tomlin & Borden 1997a,b). Alfaro and Ying (1990), Sahota *et al* (1994) and Tomlin and Borden (1997a,b) have identified Sitka spruce (*Picea sitchensis* (Bong) Carr.) clones resistant to attack by *Pissodes strobi* (Peck). However, it is not clear whether a tree's escape from attack is due to its resistance, more attractive neighbours, or to chance (Sahota *et al* 1994). Lieutier *et al* (1997b) studied the maturation feeding of *Hylobius abietis* on Scots pine clones and found a significant correlation between the concentration of an acetophenone glycoside and the amount of weevil damage. Bois *et al* (1997) observed variations in the resistance of scots pine clones to the fungus *Leptographium wingfieldii* Morelet associated with *Tomicus piniperda* attack. These studies are encouraging when investigating *P. radiata* seedling resistance to *H. ater* in New Zealand.

Traditionally, forestry practices in New Zealand have focused on breeding *P. radiata* seedlings that have superior growth qualities (i.e. fast growing trees with superior form and wood quality) (Burdon & Bannister 1985, Carson 1986, Carson & Inglis 1988, Burdon 1992, Jayawickrama *et al* 1997). If a plant has limited resources, selection for faster growth may be at the expense of tree resistance (Lorio & Sommers 1986, Price 1991, Hermes & Mattson 1992, Tuomi 1992, Weis & Campbell 1992). Resistance is thought to be polygenic (Tomlin & Borden 1997a). While the resistance of *P. radiata* to diseases such as *Sphaeropsis sapinea* (Burdon 1992) has been considered, tree resistance to insect attack has received little attention. Traits such as terpene composition have a high degree of genetic heritability (Burdon 1992). Terpene composition may play a role in the resistance of seedlings to *H. ater* attack (e.g. Paine *et al* 1997). Identifying seedling types with resistant qualities may be of value to forestry in New Zealand.

This study aims to determine whether there are differences in genetic resistance to *H. ater* attack between different *P. radiata* seedling types.

The objectives for this study were to:

- Determine whether *H. ater* showed a preference toward less resistant seedlings prior to attack
- Evaluate differences in seedling types in their resistance to *H. ater* feeding attacks

5.2 Methods

Site 34 (refer to Chapter Two) was selected in August 1999 as seedlings were predicted to be at high risk from *H. ater* attack. *H. ater* colonisation activity was high in this site following harvesting at the end of summer 1999. Subsequent larval populations were likely to overwinter and emerge to feed on newly planted seedlings the following summer. Bark was removed from a few stumps to assess *H. ater* larval populations prior to planting the trial. The presence of relatively high numbers of *H. ater* larvae indicated that the site was suitable, and that any seedlings planted in this site were likely to be attacked by *H. ater*.

Six *P. radiata* seedling types were chosen. No information with regard to the genetic history of the seedlings was available, except that the seedlings were from unrelated families (S. Downs, personal communication). Of the six seedling types, four were grown from seed and two were propagated from cuttings. There were no size differences between the seedling types planted.

The seedlings were planted in a randomised block design. Each block consisted of one row of each of the six seedling types. Twenty seedlings were planted in each row at 1m intervals. Rows were planted 3m apart. A distance of at least 15m separated each block. Five blocks of seedlings were planted. Seedlings were planted during winter (September) 1999.

During late January (2000) seedlings were accidentally sprayed with a herbicide. Five kilograms per hectare of Trounce and Pulse were applied from the air. The herbicide application was intended as a routine pre-planting weed control operation. Following the application of the herbicide (February 2000), seedlings were removed and inspected for

evidence of *H. ater* attack. Seedlings would probably have been killed by this herbicide application. Even though seedlings were removed prior to the dominant period of *H. ater* emergence (Chapter Two), feeding attacks had already taken place. At the time of sampling, effects of the herbicide on seedlings were not observed. It was assumed that all seedlings in the trial would have been exposed to equal amounts of the herbicide, and that the herbicide would effect seedlings equally. However, the study was potentially compromised by the application of the herbicide. There may have been a differential effect of the herbicide on seedling types, and this may have influenced the behaviour of *H. ater*. The results presented below will be examined for conditional conclusions.

Seedlings were destructively sampled using the methodology described in section 3.1.2. Seedlings were removed from the ground and were observed for evidence and severity of *H. ater* attack.

The severity of *H. ater* attack was recorded as follows (refer to section 3.1.2 for a full description):

0= No attack

1= Mild attack

2= Moderate attack

3= Severe attack

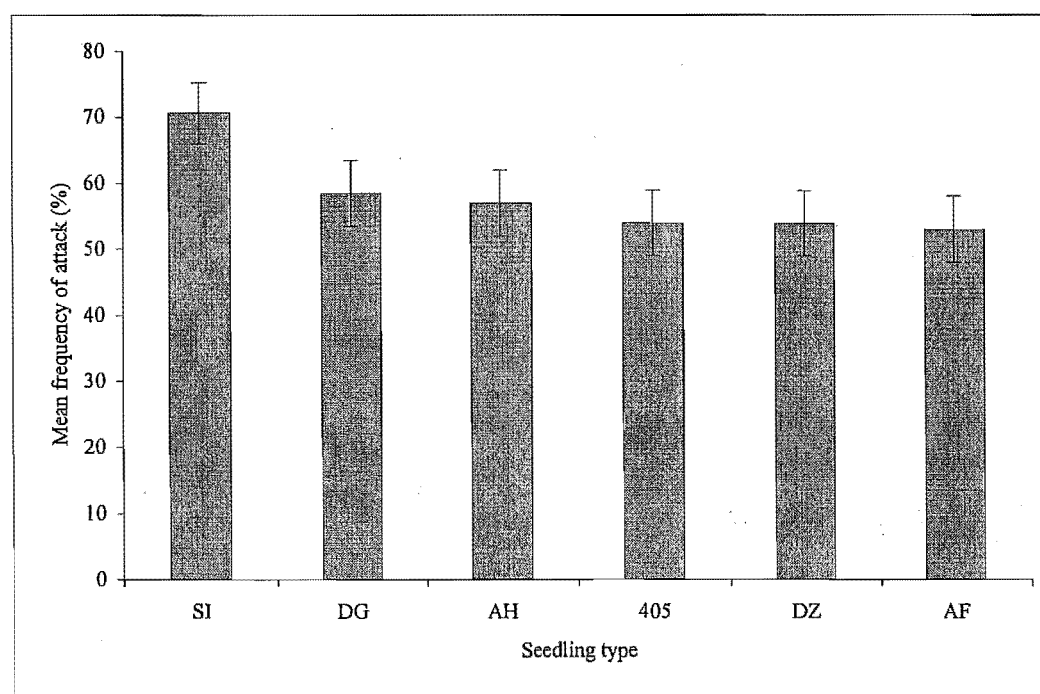
Non-parametric chi-square tests were used to investigate differences in the frequency of attack between seedling types, using the statistical package SAS (PROC FREQ, Version 6.12 for Windows, SAS Institute 1996). Analyses of variance (ANOVA) were used to investigate differences in the severity of *H. ater* attack between seedling types, using the statistical package SAS (PROC GLM, Version 6.12 for Windows, SAS Institute 1996). Pair-wise multiple comparisons were conducted using Duncan's multiple range test to determine the nature of the differences detected by ANOVA.

5.3 Results

Attack

The frequency of attack by *H. ater* was not different between seedling types ($X^2_{(1)} = 8.980$, $P > 0.05$, Figure 5.1). This indicates that *H. ater* did not make a selection of seedling type prior to feeding. The mean frequency with which seedling types were attacked ranged from 53% to 71%. There was a difference in the frequency of attack between blocks ($X^2_{(4)} = 85.618$, $P < 0.001$), indicating *H. ater* attack was variable within the site.

Figure 5.1 Mean frequency of attack by *H. ater* on different seedling types.

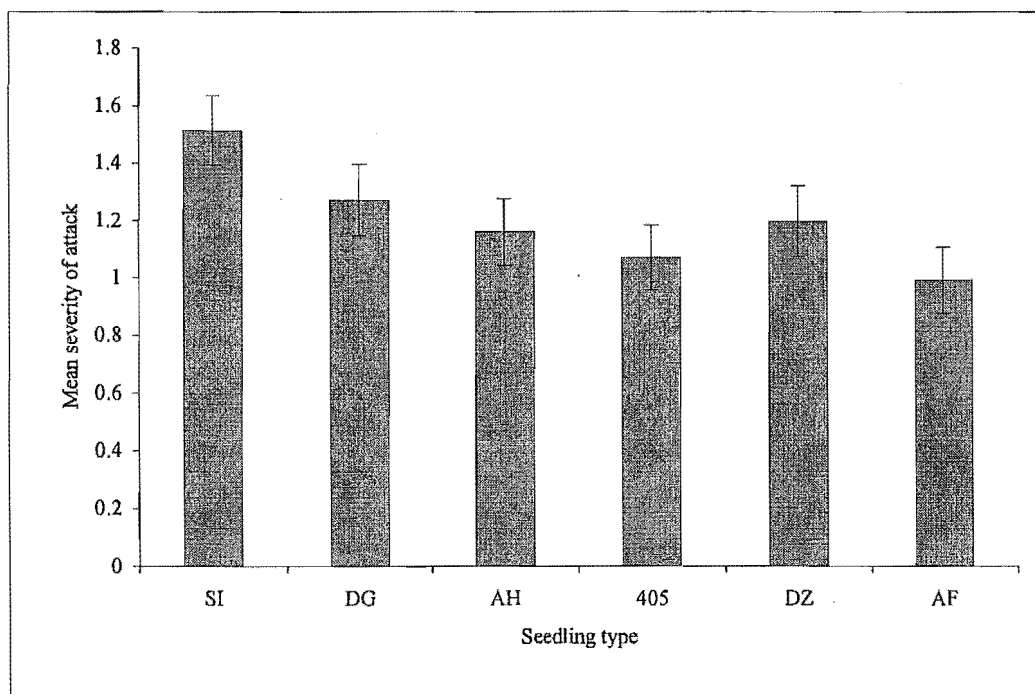


Severity of attack

The severity with which *H. ater* attacked seedlings was different between seedling types ($F_{(5,570)} = 2.86$, $P < 0.05$, Figure 5.2). This indicates that some seedling types responded better to *H. ater* attack and were more resistant. Wounds resulting from attacks on resistant seedlings were smaller than wounds on less resistant seedling types. Smaller wounds on resistant seedlings were possibly due to beetles abandoning attacks when encountering resistant seedlings. Alternatively, attacking beetles may have continued to feed, but at a reduced rate compared with less resistant seedlings. Mean severity of attack was different between blocks ($F_{(4,570)} = 28.84$, $P < 0.001$) indicating *H. ater* attack was

variable within the site. The interaction between seedling types and blocks was significant ($F_{(20,570)} = 1.60$, $P < 0.05$), indicating that relative differences in attack between seedlings types was not consistent across blocks. This variation was accounted for in the design of this study.

Figure 5.2 Mean severity of the attack on different seedling types by *H. ater*.



Results from the Duncan's multiple range test indicate that seedling types fell into two overlapping groups, based on resistance to attack (Table 5.1). Seedling types SI and DG were attacked by *H. ater* with the greatest severity. Figures 5.3 to 5.8 show that SI and DG were severely attacked more frequently than other seedling types. The remaining seedling types (including DG) were attacked with less severity than the SI and DG group (Table 5.1). The frequency with which seedling types in this group were severely attacked was less than those observed for the SI and DG grouping (Figures 5.3 to 5.8).

Table 5.1 Results from pairwise comparisons using Duncan’s multiple range test: Severity of the attack by *H. ater* on different seedling types.

Seedling type	Mean severity of attack	Number of seedlings sampled
SI	1.52 _a	99
DG	1.27 _{ab}	99
DZ	1.20 _b	102
AH	1.16 _b	100
405	10.7 _b	100
AF	0.99 _b	100

Means with the same letter are not significantly different (alpha= 0.05).

Figure 5.3 The frequency of seedling attack by *H. ater* for seedling type SI.

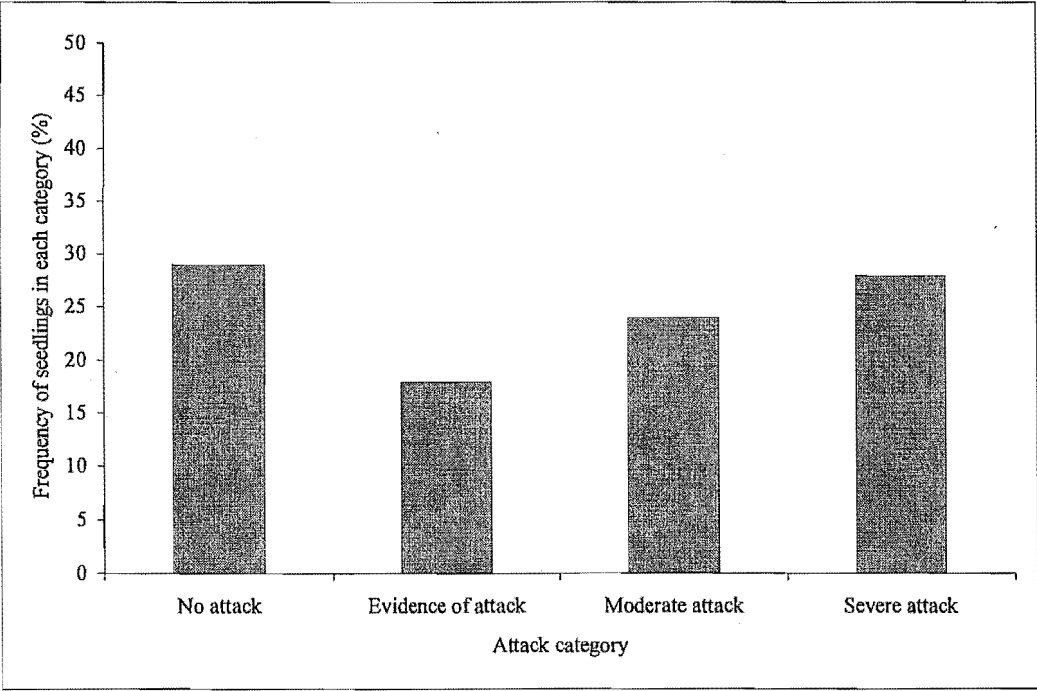


Figure 5.4 The frequency of seedling attack by *H. ater* for seedling type DG.

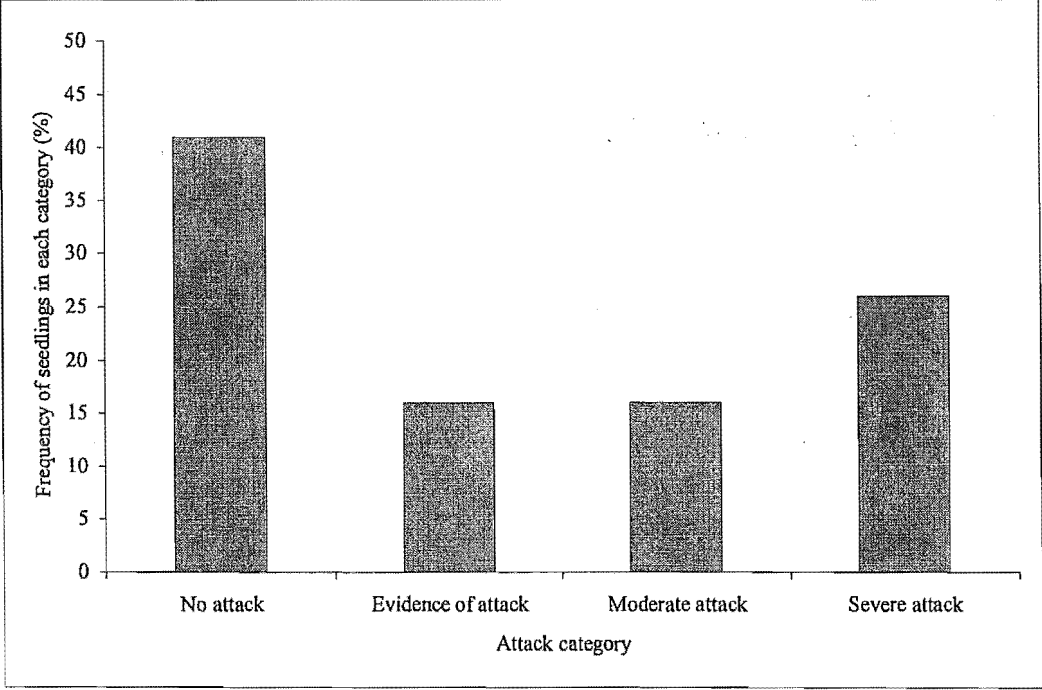


Figure 5.5 The frequency of seedling attack by *H. ater* for seedling type DZ.

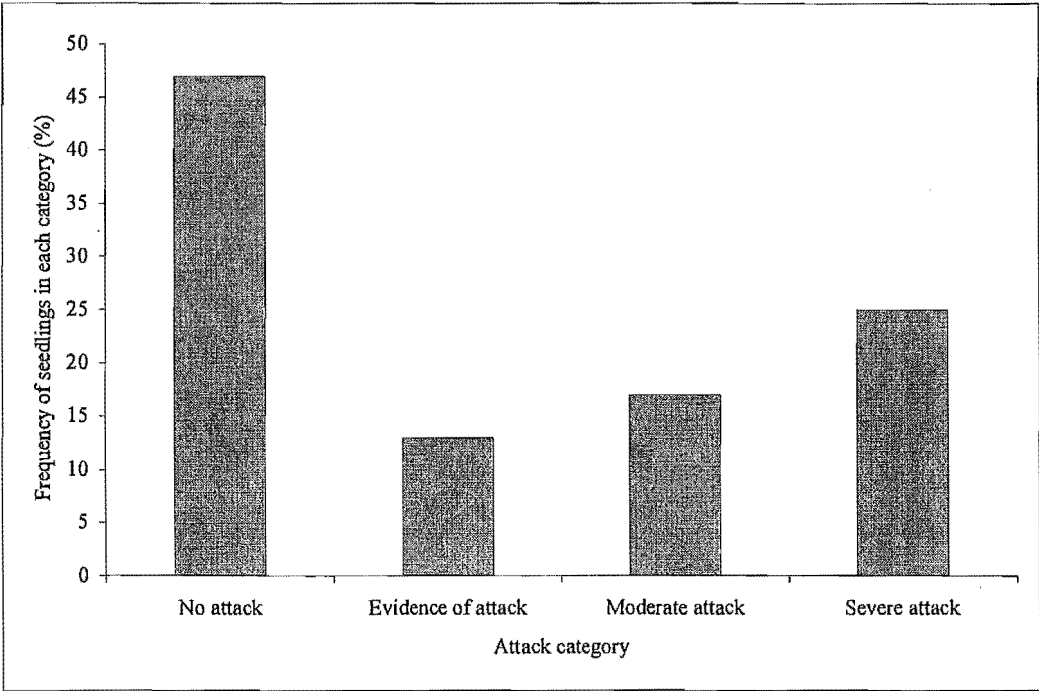


Figure 5.6 The frequency of seedling attack by *H. ater* for seedling type AH.

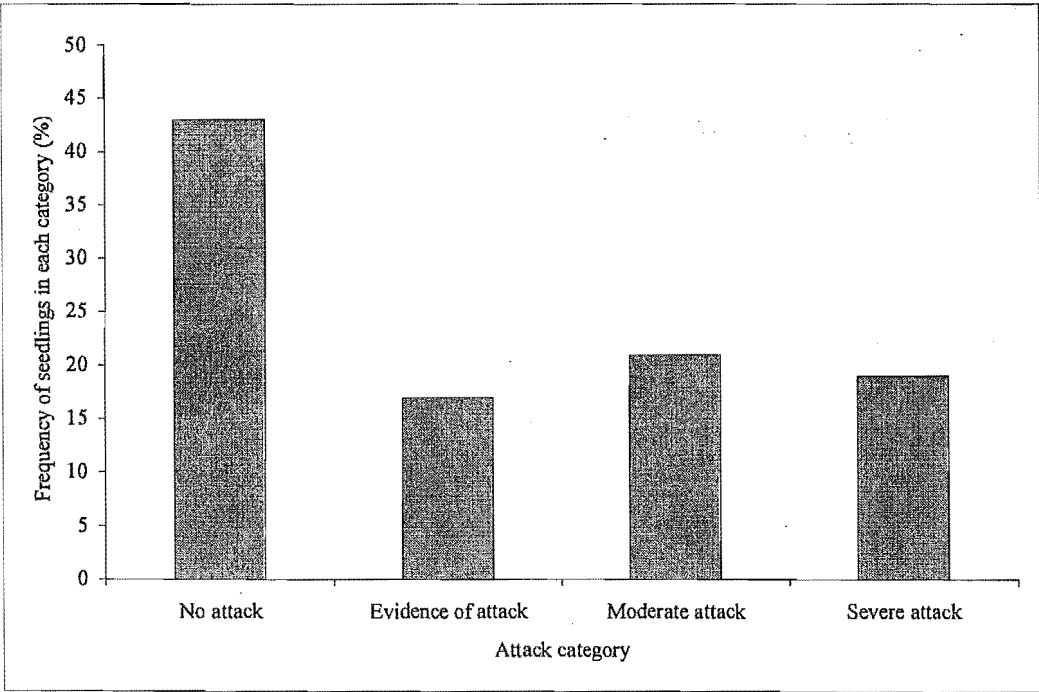


Figure 5.7 The frequency of seedling attack by *H. ater* for seedling type 405.

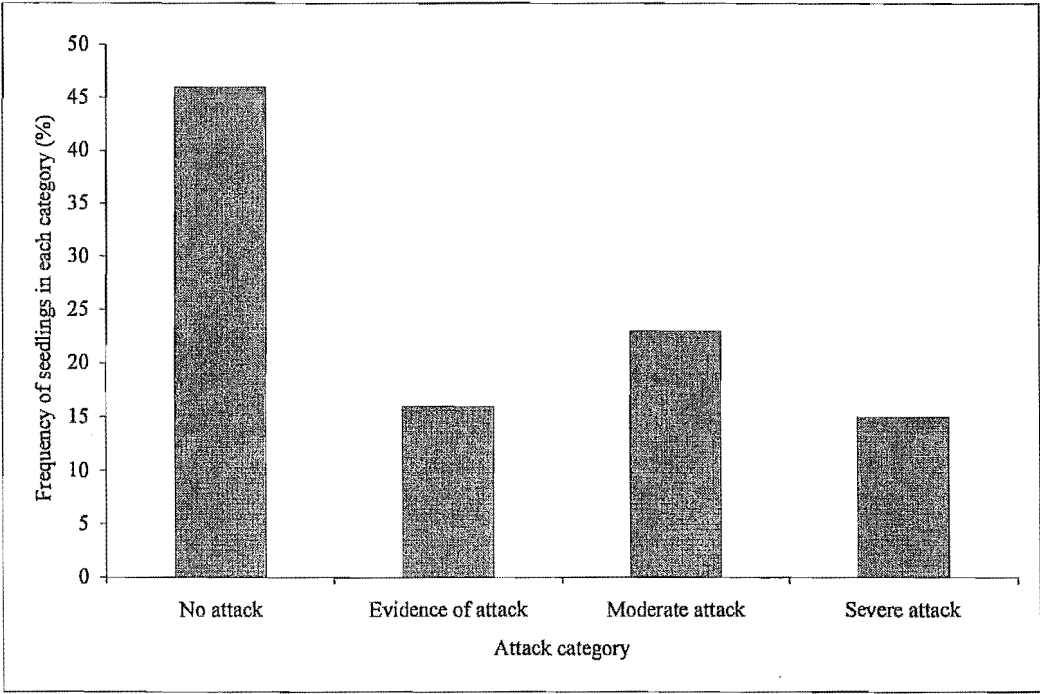
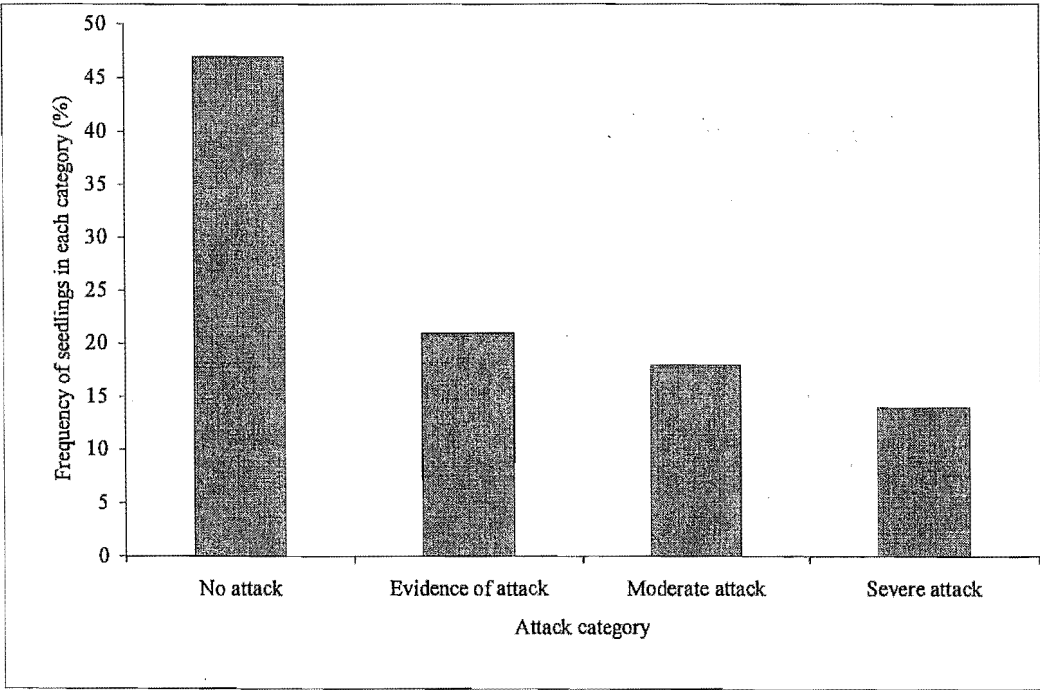


Figure 5.8 The frequency of seedling attack by *H. ater* for seedling type AF.



5.4 Discussion

The results show that seedling types may be differentiated by their response to *H. ater* attack. Those seedling types that were attacked with greater mean severity were likely less resistant to *H. ater* attack. This indicates that selection may have operated on different seedling types (Hodges *et al* 1979). Similar observations were made for the response of Scots pine to attack by *Hylobius abietis* (Lieutier *et al* 1997b) and the white pine weevil (Tomlin & Borden 1997a,b). Host plant defences are thought to be lowest during the period of establishment, when a plant is growing vigorously or when conditions are good for growth (Smalley *et al* 1993). *H. ater* attacks seedlings during maturation feeding in the first year following planting (Chapter Three). Any factor that may compromise seedling health during establishment (i.e. stress) may reduce its ability to recover from attack (Örlander & Nilsson 1999). The ability of seedlings to resist attack at this time is of major importance to the forest industry. As seedlings may also be less resistant to attack during periods of good growth (Hermes & Mattson 1992, Tuomi 1992, Smalley *et al* 1993), it may be even more important that resistant seedlings are planted in high-risk areas. These areas may be correlated with good growing conditions.

Other studies investigating bark beetle (and weevil) attack on trees have reported similar differences in response to attacks between hosts from different genetic backgrounds (e.g. Alfaro & Ying 1990, Alfaro 1997, Lieutier *et al* 1997b, Tomlin & Borden 1997a,b). Selander *et al* (1990) suggest that naturally regenerating Scots pine seedlings are more resistant to attack by *Hylobius abietis* than planted seedlings. Mason (1971) and Hodges *et al* (1979) suggest that genetic differences in host oleoresin qualities are strong resistance characteristics. The large variation in the oleoresin between the different families from which the current New Zealand *P. radiata* genotypes have been selected (Burdon *et al* 1992b) indicates great potential for developing resistant seedlings. The high heritability of terpene composition makes monoterpenes good indicators of genetic identity (Burdon *et al* 1992a). This may help make resistant traits from different families more recognisable and indicates that some resistant traits may be easily selected (i.e. those influenced by oleoresin characteristics).

This study has identified two seedling types resistant to attack by *H. ater*. However, further research is required to investigate differences between a wider range of seedling

types to find those that are most resistant. Resistant seedlings should be tested in a controlled environment to determine whether differences in resistance characteristics might be substantial enough to warrant use in forestry operations.

Aggressive bark beetles which attack mature trees may be overwhelmed by host defences and the attacking beetle is often killed (Raffa & Berryman 1983, Paine *et al* 1997). However, the death of *H. ater* adults attacking *P. radiata* seedlings in New Zealand was rarely observed (personal observation) despite seedlings being severely wounded and responding with substantial resinous exudation. The seedling response to attack does not completely overwhelm the beetle. Defence mechanisms may operate by forcing *H. ater* to abandon its feeding attempt and search elsewhere for food material. Alternatively, beetles may continue to feed at a reduced rate. Alfaro *et al* (1996) suggest the response to attack is faster for more resistant seedlings. Newly emerged *H. ater* adults attack seedlings during maturation feeding. The amount of feeding required for a beetle to reach maturity is not known. Beetles disperse from a host following a successful attack. The amount of feeding required to reach maturity and the corresponding feeding behaviour should be investigated.

While it was not possible to investigate the results of *H. ater* attack on the mortality of different seedling types in this study, mortality would probably correlate with severity of attack. If this study was sampled in May (2000) as intended, seedlings would have been subjected to greater levels of attack.

The results show that the frequency of attack did not differ between seedling types and indicate that *H. ater* selected seedlings at random. It is likely that host acceptance or rejection was made following the sampling of a seedling by *H. ater*. Severe attacks may have been due to greater numbers of feeding beetles, as beetles may be attracted to wounded seedlings (Tilles *et al* 1986a, Zagatti *et al* 1997). The volatiles released when a beetle continues feeding after accepting a less resistant host may attract other feeding beetles, whereas additional beetles may not be attracted to a resistant seedling after the initial attack has been abandoned.

Milligan (1971a) tested the hypothesis that seedling health may be important in resisting successful attack by *H. ater*. Prior to experiments by Milligan (1971a) it was believed that *H. ater* only attacked weak seedlings. Milligan (1971a) refuted this and suggested that

although weak seedlings were attacked first, it did not follow that those more vigorous plants would not be attacked if they were the only plants available. In this study, 50-70% of seedlings were attacked by *H. ater*. The results indicate that seedlings planted in high-risk sites are likely to be attacked regardless of their resistance attributes. The resistant and less resistant seedlings were attacked with equal frequency in this study. However the severity of attacks was greater upon less resistant seedling types. As the risk of invasion by sapstain fungi increases as severity of attack increases (Chapter Four) it may be beneficial to plant resistant seedlings in high-risk sites. While the frequency with which seedlings are attacked may not decrease, the effect of attack to seedling health may be less damaging.

Milligan (1971a) did not indicate whether seedlings stressed by environmental factors were more likely to be attacked before unstressed seedlings. He did show that unstressed seedlings were attacked as well as stressed ones. This study did not address whether *H. ater* were able to select stressed seedlings and confirms observations made by Milligan (1971a) that more resistant seedlings are attacked as well as less resistant ones. Other studies have reported that bark beetles may select weak or stressed trees (e.g. Heikkinen 1977, Hines & Heikkinen 1977, Tilles *et al* 1986a,b, Lindelöw 1992). Further research is required to fully understand the behaviour of *H. ater* prior to attack. The feeding behaviour of *H. ater* should be observed in a controlled environment, to determine whether beetles select less resistant seedlings, or whether attack is a chance event (e.g. Alfaro & Ying 1990, Sahota *et al* 1994).

Information on the genetic history of the seedling types planted in this trial was unavailable (the seedling types were reported to be from unrelated families). Two of the seedling types consisted of material propagated from cuttings, while the remaining four seedling types were raised from seed. This study did not show any relationship between cuttings and seedlings, and attack by *H. ater*. There may be differences in resistance to *H. ater* attack between seedlings and cuttings, or between other propagation techniques (Selander *et al* 1990, Selander 1993). Tomlin and Borden (1997b) indicate that Sitka spruce resistance to attack by *Pissodes strobi* (Peck) was correlated with bark thickness and the density of outer resin canals. Larger seedlings with thicker stems have been shown to be more resistant to attack by *Hylobius abietis* (Selander *et al* 1990, Selander 1993, Örlander & Nilsson 1999). There are differences with respect to bark thickness between *P. radiata* seedlings and

cuttings (Burdon & Bannister 1985). Characteristically, *P. radiata* cuttings show greater maturity and grow faster compared with seedlings (Burdon & Bannister 1985). Further research is required to evaluate seedlings from different propagation techniques (i.e. seedlings vs. cuttings) that have similar (or identical) genetic histories. If superior resistance is identified with a particular propagation technique, then this may be valuable when developing strategies to manage the consequences of *H. ater* attack in high-risk areas.

The trend in New Zealand has been to select for fast growing trees with superior wood qualities (Burdon & Bannister 1985, Carson 1986, Carson & Inglis 1988, Burdon 1992, Jayawickrama *et al* 1997). It is likely that this has been at the expense of other plant characteristics. While theories of plant defence are complicated, the basic genetic trade off between growth and defence is widely held (Price 1991, Hermes & Mattson 1992, Tumoi 1992, Weis & Campbell 1992). It is possible that selection for superior growth qualities comes at the expense of resistance to herbivory. By comparing *H. ater* attack between seedling types that have undergone extensive selections for faster growth with older seedling types that have not been subjected to extensive selection, it may be possible to evaluate the extent to which selection has influenced seedling defences. If significant differences are found, it may be possible to utilise the original genetic material to breed seedlings with superior resistance characteristics.

Planting seedlings that are resistant to attack may result in a reduction in the severity of attack. While minimising mortality resulting from *H. ater* attack is currently the primary objective in forestry operations, the effects of sub-lethal attack by *H. ater* on seedlings may be as important (Chapter Four). As the likelihood of invasion by sapstain fungi increases as severity of attack increases (Chapter Four), planting resistant seedlings in higher risk sites would be advantageous. Genetic traits for increased resistance to insect attack may also be correlated with increased resistance to other organisms (e.g. sapstain fungi) (Bois *et al* 1997). Resistant seedlings may resist severe *H. ater* attack and therefore reduce the potential for invasion by fungi (or other organisms). If fungi are able to directly invade a seedling, resistant seedlings may be better at containing or resisting this invasion (Bois *et al* 1997 Lieutier *et al* 1997a, Ranta *et al* 2000). Further research is required to investigate any relationship between seedling resistance to *H. ater* attack and invasion by fungi.

This study presents data on seedling resistance to *H. ater* attack. It is possible to identify *P. radiata* seedling material with resistance characteristics. Further research may identify seedling material that has resistant characteristics with growth losses that are not economically significant. The results presented here indicate that this is an interesting area of *H. ater* research. Further research should:

- Demonstrate that differences found between these genotypes are consistent with other trials
- Determine the effect of *H. ater* attack on the mortality of different seedling types
- Assess resistance to *H. ater* in a greater range of seedling material
- Assess the effect of different propagation techniques on seedlings resistance
- Determine whether *H. ater* is able to select stressed seedlings prior to attack
- Investigate potential 'trade-offs' between resistance and growth for seedlings with increased resistance to *H. ater*
- Investigate whether seedlings that demonstrate superior resistance to *H. ater* also show superior resistance to other organisms (i.e. sapstain fungi)
- Determine how feeding rate is related to bark tissues
- Investigate whether feeding wounds make seedlings more attractive to other beetles
- Investigate how much feeding is required by *H. ater* to satisfy maturation requirements
- Determine the level of feeding required to kill seedlings

6. THE ORIENTATION OF *H. ater* AND *H. ligniperda* TO HOST VOLATILES AND BROAD-SPECTRUM ATTRACTANTS

6.1 Introduction

The differing life histories of the bark beetle groups described in Chapter One are reflected in the colonisation strategy each group employs to penetrate the host (Raffa *et al* 1993). While most scolytids use a combination of tree and insect produced volatiles in host finding and colonisation, the role of volatiles varies (Raffa *et al* 1993). In general, primary species use volatiles as cues for aggregation when overwhelming hosts (Rudinsky 1962, Wood, D.L. 1982, Raffa & Berryman 1983, Raffa *et al* 1993). Non-aggressive species are attracted to host volatiles and use these to select hosts (Rudinsky 1962, Wood, D.L. 1982, Raffa & Berryman 1983). These 'primary' cues may also elicit 'secondary' (i.e. pheromone) attraction (Raffa *et al* 1993).

Wood, D.L. (1982) classified the process of host colonisation into four phases: dispersal, selection, concentration and establishment. Dispersal begins with emergence from the brood host and concludes with a response to volatiles and/or pheromones (Wood, D.L. 1982). Little is known about the dispersal phase of scolytid colonisation strategies. Past studies have investigated the amount of energy resources, effects of physical stimuli on the preparation and initiation of flight, and the responses of new emergents to volatiles (Wood, D.L. 1982, Raffa *et al* 1993). Host selection is partly dependent on flight capacity and behaviour, as these determine where new hosts are found (Gara & Vité 1962). Studies of flight behaviour are an important aspect of understanding bark beetle biology. However, such studies are confounded by the logistics of observing small animals in flight and problems associated with trapping techniques (Gara & Vité 1962). Consequently, experimental attempts have failed to meet researchers expectations (Gara & Vité 1962).

Emerging beetles are positively photoactive and negatively geotrophic (Atkins 1966, Zethner-Møller & Rudinsky 1967, Raffa *et al* 1993). Scolytids have the potential for flights of some distance. For example, *Ips typographus* up to 19 km (De Jong & Sabelis

1988), *Hylobius abietis* up to 100 km (Solbreck 1980, Nilssen 1984). However most fly shorter distances, influenced by attraction to volatiles (Rudinsky & Zethner-Møller 1967, Wood, D.L. 1982, Tilles *et al.* 1986a,b, Lindelöw 1992, Lindelöw *et al.* 1992, Raffa *et al.* 1993). Flight activity and duration are also influenced by season, energy and lipid content, quality and quantity of larval food (Rudinsky & Vité 1956, Atkins 1966, Birch 1978, Wood, D.L. 1982, Raffa *et al.* 1993). Variation in the duration of flight may represent an optimal strategy for exploiting temporary resources (Gara & Vité 1962, Atkins 1966, De Jong & Sabelis 1988) and for the expansion of local infestation (Raffa *et al.* 1993). Flight is likely to be required before beetles respond to volatiles (Graham 1959), or a response to volatiles may be required for emergence (Wood, D.L. 1982).

One advantage of longer distance dispersal is the increase in genetic material a population may gain through outbreeding (Borden 1982, Wood, D.L. 1982, Raffa *et al.* 1993). Inbreeding is likely to be common for many species (Raffa *et al.* 1993). However, the further individuals disperse from the population, the more dilute the population becomes (De Jong & Sabelis 1988). Two choices are available for an individual: remain with the population and attack healthy hosts, or disperse and find other suitable (less healthy) host trees. Individuals with low energy reserves may be best staying with the population (and risk interspecific competition etc.), while it may be better for those with high energy reserves to exploit resources further away (De Jong & Sabelis 1988). Long periods of dispersal with flights over great distances appears to be the prevalent strategy when a population is under endemic conditions and suitable hosts may be scarce. Concentrated fights over short distances may be more characteristic of a population under epidemic conditions with greater numbers available to overwhelm hosts (Gara & Vité 1962).

Host selection begins with a response to host stimuli prior to landing and results in sustained feeding in the phloem (Wood, D.L. 1982). Host selection may either be a random or non-random process (Birch 1978, Borden 1982, Wood, D.L. 1982, Raffa *et al.* 1993). Pearson (1931) suggests that susceptible trees are selected by direct orientation to volatile compounds after observing tree death as being non-random. The processes that drive selection for certain trees have been the focus of considerable debate. Two hypotheses are predominant in the current literature: host selection as a random process, and primary attraction to host volatiles (Rudinsky 1962, Borden 1982, Wood, D.L. 1982, Raffa *et al.* 1993).

There is evidence to support both hypotheses, which may depend on the breeding strategy of different species (Wood, D.L. 1982, Raffa *et al* 1993, Tunset *et al* 1993). Distinguishing between these hypotheses can be complex (Birch 1978, Tunset *et al* 1993). Selection, attack and colonisation are events which form a continuum, rather than being distinct through time (Birch 1978). In addition, the presence of aggregation pheromones and kairomones may interfere when attempting to identify the cues a species may be responding too (Tunset *et al* 1988). However, random host selection is thought to be the attack strategy of several primary species (Raffa & Berryman 1983, Raffa *et al* 1993, Tunset *et al* 1993).

With random selection, a species lands at random on potential host trees. Only after landing is a decision made to accept a host (Wood, D.L. 1982, Raffa & Berryman 1983, Tunset *et al* 1993). Raffa *et al* (1993) suggests that visual cues are used by these species, and the rejection or acceptance of a host is made after a close-range evaluation. Upon the selection of a suitable host tree primary and aggressive species employ aggregation pheromones or other chemical stimuli to attract (secondary attraction) others. While randomly selecting a host may seem hazardous for a species searching for a limited resource, this in combination with secondary attraction is very effective (Raffa *et al* 1993) and has been documented by Gara and Vité (1962), Löyttyäniemi and Hiltunen (1976) and Berryman (1972). Over 90% of trees are visited by beetles (Raffa & Berryman 1983, Raffa *et al* 1993).

Vasechko (1988) suggests that the following contradict the random attack concept: Certain species that were previously claimed to produce pheromones do not. Some species (e.g. *Dendroctonus frontalis*, *D. ponderosae* and some *Ips* species) previously claimed to attack any tree, show preferences toward weaker trees. Other species that were claimed to visit potential hosts at random show preferences for weak trees, wounds and volatiles.

Primary attraction allows an individual to detect a suitable host in flight using chemical and or visual cues (Wood, D.L. 1982, Raffa *et al* 1993, Tunset *et al* 1993). While secondary and non-aggressive bark beetles commonly employ this host selection technique (Raffa *et al* 1993), some tree killing scolytids also may use it (Wood, D.L. 1982). Non-aggressive or secondary species that respond to host volatiles often respond to pheromones. However, not all species are capable of producing and employing

pheromones (Wood, D.L. 1982, Tunset *et al* 1993). Once a host has been selected, most of a population will respond to pheromones released by the pioneer beetles, host volatiles (Wood, D.L. 1982, Tilles *et al* 1986a,b, Lindelöw 1992, Raffa *et al* 1993), or possibly visual cues (Eidmann *et al* 1991). However, they do not encounter the same host defences as primary or aggressive species (Wood, D.L. 1982).

As secondary and non-aggressive beetles do not have the ability to overwhelm healthy or living hosts, colonising beetles must be able to locate and detect the most susceptible hosts. Attractants may be effective over long distances (i.e. 600 m (Vité & Gara 1962)). Species that orientate by primary attraction must also utilise this strategy to orientate toward the correct breeding substrate (Schroeder 1988, Schroeder & Lindelöw 1989, Lindelöw *et al* 1992). Evidence for primary attraction has been supported by field and laboratory observations where species select baited over unbaited traps (Löyttyneimi & Hiltunen 1976, Tunset *et al* 1988, Eidmann *et al* 1991, Tunset *et al* 1993). Evidence of primary attraction has been widely documented (Vité & Gara 1962, Heikkinen & Hrutfiord 1965, Moeck 1970, Hines & Heikkinen 1977, Visser 1986, Witcosky *et al* 1987, Schroeder 1988, Chénier & Philogène 1989, Schroeder & Lindelöw 1989, Eidmann *et al* 1991, Lindelöw 1992, Lindelöw *et al* 1992, Lindelöw *et al* 1993, Tunset *et al* 1993, Brattli *et al* 1998).

Injured or stressed trees differ in chemical composition from healthy trees. Bark beetles often respond to olfactory cues related to these differences (Heikkinen 1977, Hines & Heikkinen 1977, Tilles *et al* 1986a,b, Lindelöw *et al* 1992). For example, the terpene composition of phloem may provide information about the condition of a potential host species (Wood, D.L. 1982, Lindelöw 1992). Plant stress and injury by the fungus *Leptographium wagnerii* initiates the production and release of insect attractant volatiles from Douglas-fir (Witcosky *et al* 1987). Attraction to deterioration products is thought to be a dominant cue to help beetles distinguish the most appropriate host (Lindelöw 1992, Tunset *et al* 1993). Different species are attracted to different hosts and stages of host deterioration by differing release rates and combinations of compounds (Löyttyneimi & Hiltunen 1976, Schroeder 1988, Schroeder & Lindelöw 1989, Lindelöw *et al* 1992, Flechtmann *et al* 1999). Often scolytid host selection behaviour is initiated as a response to deterioration compounds released as a result of primary bark beetle attack (Tunset *et al* 1993).

The concentration phase for primary or aggressive species begins with attraction to pheromones, boring into the host plant or the release of host compounds. This phase is terminated when pheromone attraction ceases or anti-attractants are released (Wood, D.L. 1982). Little is known about the feeding behaviour prior to the release of pheromones (Wood, D.L. 1982). Host acceptance may be based on taste, short distance olfaction and chemical cues on contact (Raffa *et al* 1993). Sustained feeding indicates the presence of required chemical precursors and/or those stimulants required for pheromone biosynthesis (Wood, D.L. 1982). Raffa *et al* (1993) suggest that sustained feeding is not normally necessary to determine the status of a potential host.

Aggregation and/or sex pheromones are often employed by bark beetles during the colonisation of host material (Borden 1989). Aggregation pheromones are produced by and induce aggregation in both sexes. Sex pheromones are produced by one sex, which affects the other. Aggregation pheromones may become anti-aggregation pheromones at high concentrations. A pheromone acting with a host produced compound or which acts on another species is called a kairomone or an allomone, depending on whether it is beneficial or harmful, respectively (Borden 1982, Borden 1989).

Pheromones produced by aggressive 'primary' species are required to induce rapid aggregation in order to generate sufficient numbers to overwhelm host tree defences (Birch 1978, Alcock 1982, Borden 1982, Anderbrant 1990). Failure to attract other colonising individuals is likely to result in the death of the pioneer, or the pioneer must abandon the attack. Mortality from resinosis is inversely related to the density of the attack (Berryman & Ashraf 1970, Raffa & Berryman 1983). Successfully attacked trees are always colonised by large numbers of beetles (Raffa & Berryman 1983). The faster a pioneer can attract colonisers, the more likely it is to survive. Consequently, 'contact' pheromones have been proposed where pheromones are produced on contact with the hosts oleoresin system (Birch 1978, Borden 1982, Wood, D.L. 1982). Species of *Dendroctonus* may release pheromone components prior to host penetration. However the mechanisms of release or synthesis are not known (Wood, D.L. 1982), but indicate the urgency of these species to build numbers rapidly in order to survive tree defences (Birch 1978, Borden 1982). Less aggressive species do not have the same urgency to build numbers rapidly, and aggregation may be sex-orientated. Thus, pheromone production does not need to be

as rapid, and “frass” pheromones are produced following feeding (Vité 1967, Birch 1978, Borden 1982, Wood, D.L. 1982).

Long-range directed orientation toward a pheromone source may be either klinotatic or tropotactic. Klinotactic is the alternative sampling of either side of the body with the beetle moving in an arcing manner, with arcs reducing in size as it gets closer to the source. Tropotaxis is where information from each antenna is compared to determine the direction of the pheromone source (Borden 1982). Other stimuli, such as visual and auditory, may complement pheromone attraction and can enhance orientation toward the host tree (Birch 1978).

Once aggregation has occurred, feeding and mating may begin (Borden 1982). Most bark beetles arrive to the host as a response to pheromones or secondary attractants (Raffa *et al* 1993). In the case of primary or aggressive species, pathogenic fungi are inoculated during attacks and tree defences are exhausted (Raffa *et al* 1993). Some primary species optimise attack densities to maximise brood development whilst ensuring attack mortalities are low (Raffa *et al* 1993).

H. ater and *H. ligniperda* colonise dead tree material (Bain 1977a, Milligan 1978). Large numbers of aggregating individuals are not required to successfully overwhelm a living host to create a breeding habitat. Aggregation pheromones are not likely to be present in these species. However, it is possible that aggregation may occur as a response to sex pheromones when potential hosts are found. In second rotation forests the dominant source of host material is *P. radiata* stumps. An assumption was made in Chapter Two that both *H. ater* and *H. ligniperda* orientate toward host volatiles. Raw turpentine was used as the primary attractant in the study of the flight activity (Chapter Two), as it was thought to provide suitable attraction (Vité & Gara 1962, Phillips 1990) while being inexpensive.

In addition to evaluating the effectiveness of raw turpentine as an attractant to *H. ater* and *H. ligniperda*, this study provided an opportunity to investigate the hypothesis that these secondary bark beetle species orientate towards host volatiles. Orientation to host volatiles has been suggested for other species of *Hylastes* (Lindelöw 1992). Often attraction is increased with the presence of products of deterioration (e.g. ethanol), as these may

indicate host susceptibility (Tilles *et al* 1986a,b, Norlander 1989, Phillips 1990, Lindelöw 1992, Byers 1995, Leather *et al* 1999).

The objectives of this study were to:

- Investigate the attractiveness of a number of volatiles to *H. ater* and *H. ligniperda*, and in particular to evaluate the effectiveness of the raw turpentine used as an attractant in Chapter Two.
- Determine whether both species were attracted to the same compounds.
- Identify the best performing attractant for use in future studies.
- Investigate whether *H. ater* and *H. ligniperda* use host volatiles as the primary means of finding suitable host material.
- Evaluate whether ethanol had a synergistic affect on the attractiveness of volatiles.
- Investigate the likelihood of an aggregation pheromone or other form of pheromone attraction in *H. ater*.

6.2 Methods

Eight treatments were tested for attractiveness to *H. ater* and *H. ligniperda* in the field.

The treatments were as follows:

1= <i>Alpha</i> pinene (α -pinene)	5= Raw turpentine
2= <i>Alpha</i> pinene (α -pinene) and ethanol	6= Raw turpentine and Ethanol
3= <i>Beta</i> pinene (β -pinene)	7= Ethanol
4= <i>Beta</i> pine (β -pinene) and ethanol	8= Control (no treatment)

α -pinene

α -Pinene (2,6,6-trimethylbicyclo[3.1.1]hept-2-ene) (200ml) was supplied as a lure by Phero Tech Inc. and contained 99.2% α -Pinene.

β -pinene

β -pinene (6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane) (200ml) was supplied as a lure by Phero Tech Inc. and contained 99.2% β -pinene.

Ethanol

Ethyl Alcohol was used at a concentration of 95%.

In recent years these products (α -Pinene, β -pinene & ethanol) have been shown to be effective in detecting a broad spectrum of wood-dwelling beetles.

Raw Turpentine

Raw turpentine is a product of chemical pulping. The monoterpenes that make up the raw turpentine are distilled from chemical pulp digesters (Burdon *et al* 1992a). While the relative amounts of the monerpene constituents may vary from tree to tree (Burdon *et al* 1992a), the contents of “typical” raw turpentine from the Carter Holt Harvey Kinleith Mill was as follows (Eka Chemicals NZ Ltd).

Component	% w/w	Component	% w/w
Tricyclene	0.12	trans-b-terpineol	0.02
α -Pinene	34	α -terpineol	1.97
α -fenchene	0.14	borneol	0.5
camphene	0.83	terpinolene	0.68
β -Pinene	52.3	fenchone	0.05
3-carene	1.27	camphor	0.06
myrcene	0.55	terpene-1-ol	0.08
α - phellandrene	0.1	α -fenchol	0.54
α -terpinene	0.15	terpinene-4-ol	0.33
limonene	2.31	β -terpineol	0.03
β -phellandrene	2.02	iso-borneol	0.03
g-terpinene	0.13		

While it was not possible to control the release rates of the raw turpentine and ethanol volatiles, it was assumed that these were consistent between treatments across the study.

The attractiveness of the eight treatments to *H. ater* and *H. ligniperda* were tested in the field. A recently harvested site was selected in Kinleith Forest.

Beetles were trapped using eight funnel Lindgren (1983) traps from Phero Tech Inc. (Figure 6.1) as described in section 2.2.1.

Six blocks (rows) of the eight treatments were arranged within the site. A minimum distance of 10m separated each row of traps. Traps within each row were placed at least 5m apart. The eight treatments were randomly located in each row.

Figure 6.1 Construction of the Lindgren funnel traps.

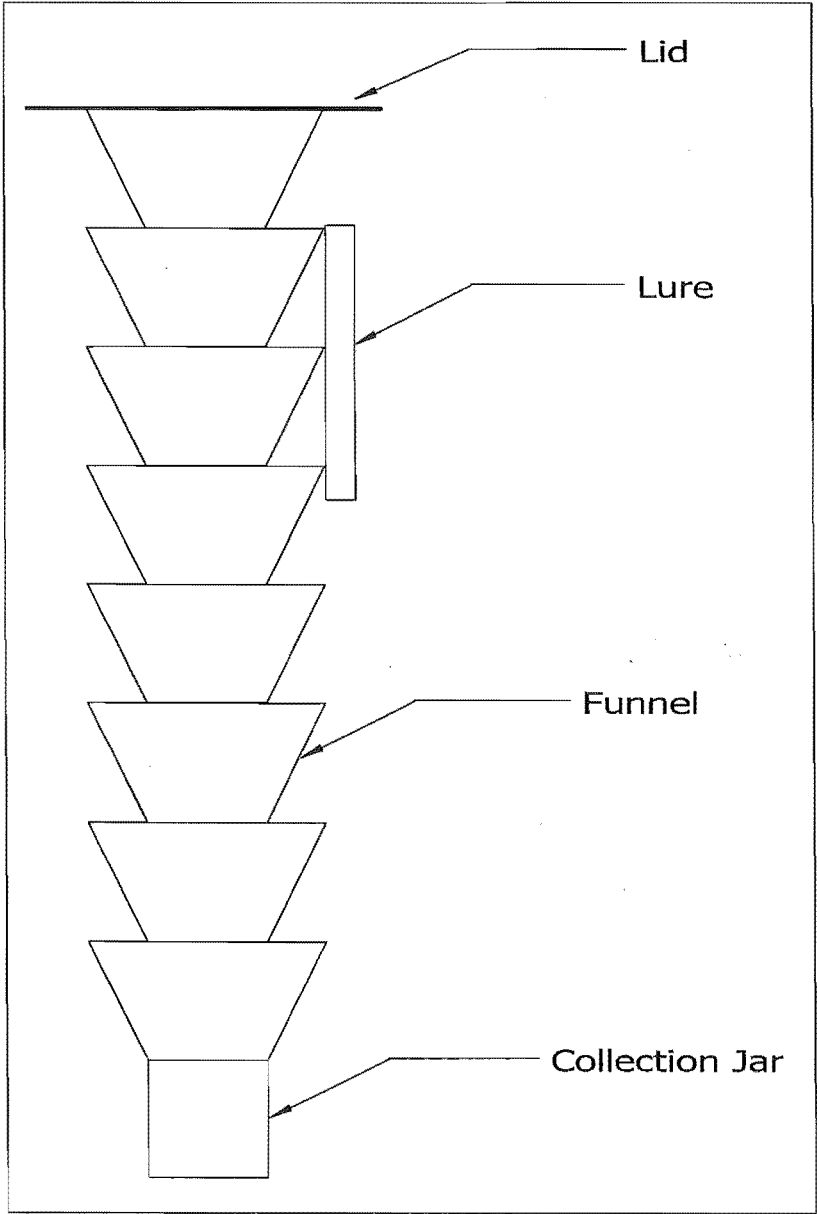
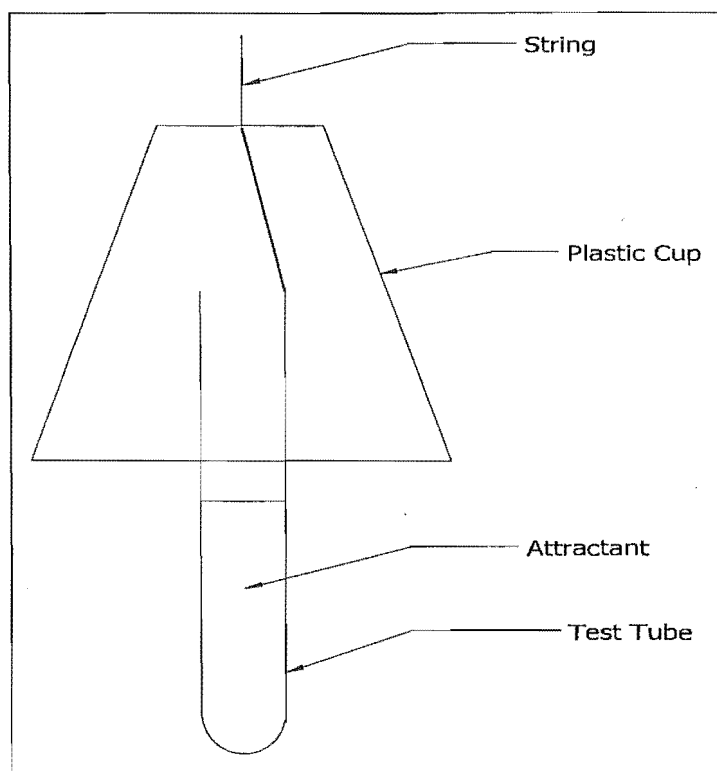


Figure 6.2 Construction of the test tube device to release raw turpentine and ethanol volatiles.



While the α - and β -pinene lures were ready to be used with traps, a method of attaching the remaining treatments (raw turpentine and ethanol) to the traps had to be devised (Figure 6.2). A lure was constructed using a 100 ml glass test tube hanging from a length of string. The string was attached to the test tube using strong waterproof plastic tape. A clear plastic cup was hung from the string over the top of the tube to prevent rain from entering the tube. The cup was hung in such a way that allowed the volatiles in the tube to circulate. Raw turpentine (50 ml) and ethanol (50 ml) were placed in separate tubes as required. The lures were hung from the top of the traps using string

Traps were placed in the field on 26 February 2000. Trapping continued until 7 April 2000. Seven collections were made from the traps at approximately weekly intervals. While the α - and β -pinene lures lasted the duration of the trial the raw turpentine and ethanol lures were replaced once during the trial. All insects were collected from the traps and were stored in plastic jars containing 70% ethanol until sorting.

Analyses of variance (ANOVA) were used to compare the attractiveness of the different treatments, using the statistical package SAS (PROC GLM, Version 6.12 for Windows, SAS Institute 1996). Pairwise multiple comparisons were conducted using Duncan's multiple range tests to determine the nature of the differences detected by ANOVA.

6.3 Results

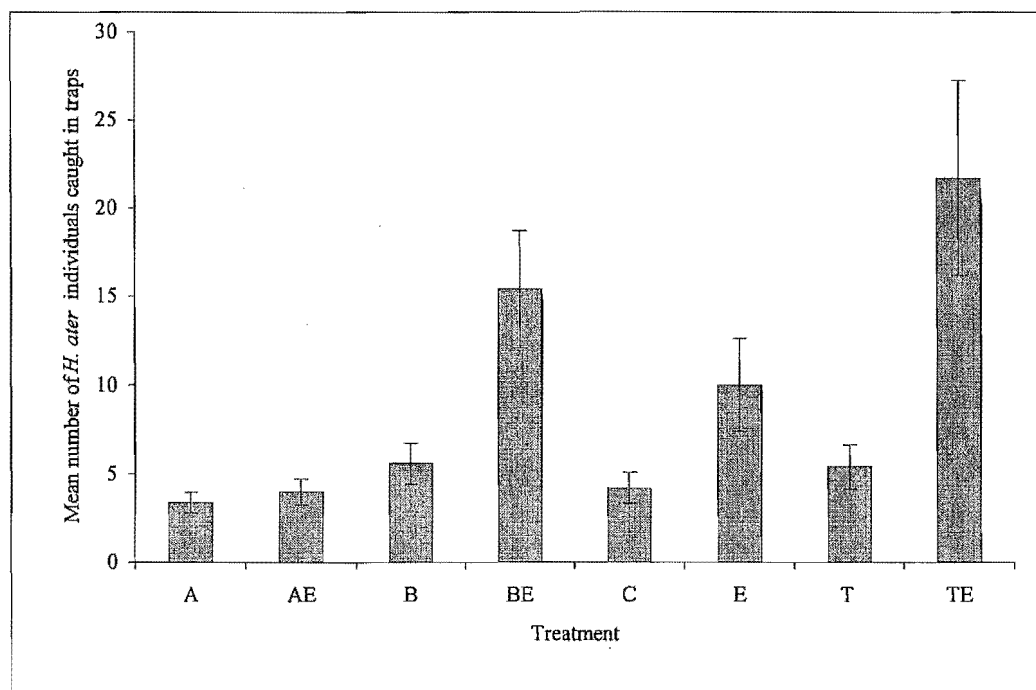
Both *H. ater* and *H. ligniperda* showed stronger preferences toward some volatiles over others. In total, many more *H. ligniperda* (25 266 individuals) were caught compared with *H. ater* (2 819 individuals) over the six week period. The difference in the number of individuals caught between the two species represents the activity of each species during the trial period (Chapter Two). The number of *H. ater* individuals caught was relatively low until the last two weeks of the trial, when the flight activity of *H. ater* increased sharply. However, numbers of both species were sufficient to demonstrate differences between treatments.

The number of *H. ater* individuals caught was different between treatments ($F_{(7,229)} = 16.93$, $P < 0.001$, Figure 6.3). Differences between treatments were assessed using Duncan's multiple range test (Table 6.1). Raw turpentine and ethanol was the most attractive treatment, followed by β -pinene and ethanol, then ethanol, β -pinene and raw turpentine. There was overlap between the third and least attractive groups, with β -pinene and raw turpentine falling into both groups. The final group contained treatments that were least attractive to *H. ater*. The results show that β -pinene, raw turpentine, α -pinene and α -pinene and ethanol were no more attractive to *H. ater* than no treatment (control). The results show a synergistic effect between ethanol and the other treatments. When ethanol is added to raw turpentine and β -pinene, the attractiveness of the combination is significantly stronger than for these components alone.

The number of *H. ater* individuals caught was different between blocks ($F_{(5,229)} = 5.14$, $P < 0.001$) and time periods ($F_{(6,229)} = 45.54$, $P < 0.001$). The significant interaction between treatments and blocks ($F_{(35,229)} = 1.87$, $P < 0.01$) indicates that the relative numbers of *H. ater* caught in treatments were not consistent across the site. Likewise, the significant interaction between treatments and time periods ($F_{(42,229)} = 5.26$, $P < 0.001$) indicates that

the relative numbers of *H. ater* caught in treatments were not consistent between the collections. These interactions represent differences in relative activity of *H. ater* across the trial area. The trial was designed to account for this variation. Despite these relative differences in activity in the trial area with respect to space and time, treatment was the dominant factor influencing the number of *H. ater* individuals caught.

Figure 6.3 Mean numbers of *H. ater* individuals caught using different treatments.



A= α -pinene, AE= α -pinene and ethanol, B= β -pinene, BE= β -pinene and ethanol, C= control, E= ethanol, T= raw turpentine, TE= raw turpentine and ethanol.

Table 6.1 Results for the pairwise comparisons using Duncan's multiple range test: Attraction of *H. ater* to different volatiles.

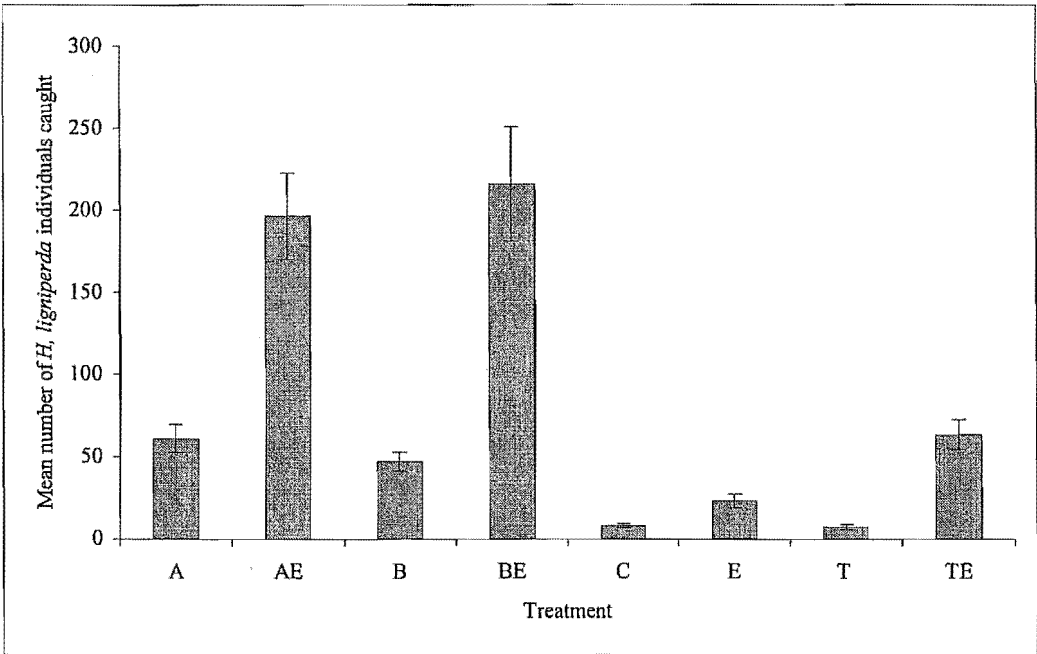
Treatment	Mean number of <i>H. ater</i> caught	Number of trap catches
raw turpentine and ethanol	21.7 _a	41
β-pinene and ethanol	15.4 _b	40
ethanol	10.0 _c	40
β-pinene	5.5 _c	42
raw turpentine	5.4 _{cd}	39
control	4.2 _{cd}	42
α-pinene and ethanol	4.0 _d	41
α-pinene	3.4 _d	40

Means with the same letter are not significantly different ($\alpha=0.05$).

Numbers of *H. ligniperda* caught differed between treatments ($F_{(7,229)}=77.42$, $P<0.001$, Figure 6.4). Differences between the treatments were assessed using Duncan's multiple range test (Table 6.2). α-pinene and ethanol, and β-pinene and ethanol were the most attractive treatments. The remaining groupings show some overlap, but the increase in attractiveness with the synergy of ethanol and the volatiles shown for *H. ater* is also apparent for *H. ligniperda*.

Numbers of *H. ligniperda* caught differed between blocks ($F_{(5,229)}=27.77$, $P<0.001$) and time period ($F_{(6,229)}=29.66$, $P<0.001$). Significant interactions between treatments and blocks ($F_{(35,229)}=6.23$, $P<0.001$) and treatments and time periods ($F_{(42,229)}=4.71$, $P<0.001$) indicate the relative numbers of *H. ligniperda* caught in treatments was not consistent across the site. However, this variation was expected and was accounted for in the trial design.

Figure 6.4 Mean numbers of *H. ligniperda* individuals caught using different treatments.



A= α -pinene, AE= α -pinene and ethanol, B= β -pinene, BE= β -pinene and ethanol, C= control, E= ethanol, T= raw turpentine, TE= raw turpentine and ethanol.

Table 6.2 Results for the pairwise comparisons using Duncan's multiple range test: Attraction of *H. ligniperda* to different volatiles.

Treatment	Mean number of <i>H. ligniperda</i> caught	Number of trap catches
β -pinene and ethanol	215.9a	40
α -pinene and ethanol	196.4a	41
raw turpentine and ethanol	63.5b	41
α -pinene	61.0b	40
β -pinene	47.1bc	42
ethanol	23.1cd	40
control	8.1d	42
raw turpentine	7.5d	39

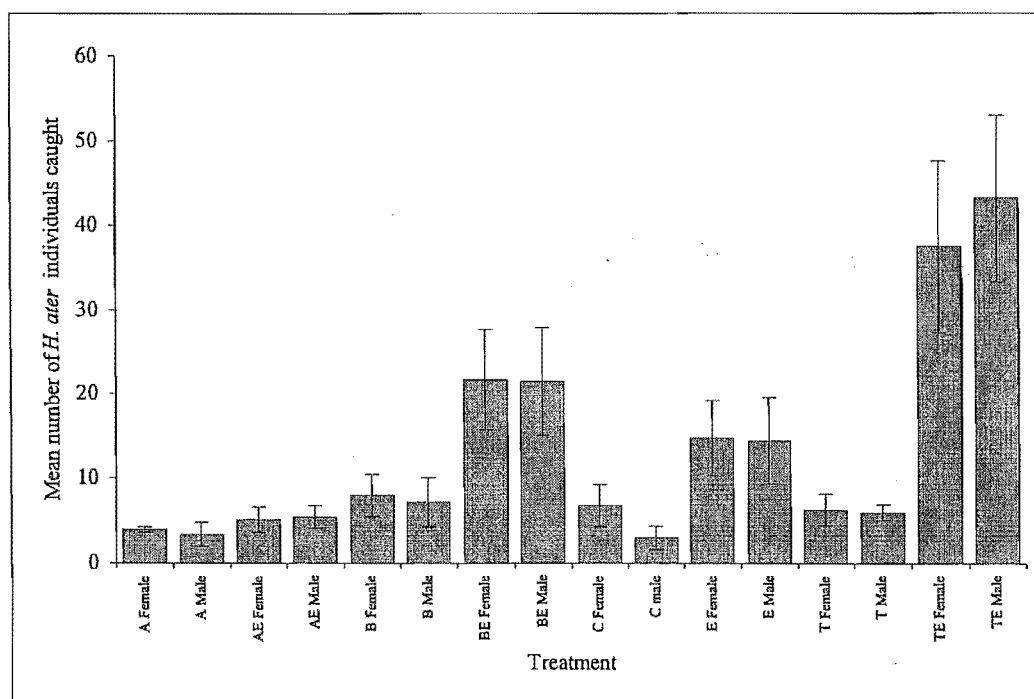
Means with the same letter are not significantly different ($\alpha=0.05$).

Sex ratios of *H. ater*

H. ater individuals caught during the final period of trapping (30 March to 7 April 2000) were examined to determine whether they were male or female on the following basis: “males are distinguishable from females by the formation of the ultimate sternite, which is less convex and has a conspicuous finely pubescent depression” (Clark 1932b). During this period, 1 244 (622 male and 622 female) *H. ater* individuals were caught (note sex determination was equivocal).

While the number of *H. ater* individuals was different between treatments ($F_{(7,78)} = 13.78$, $P < 0.001$), male and female beetles were caught in equal numbers ($F_{(1,78)} = 0.00$, $P > 0.05$). There was no significant interaction between treatments and the sex of *H. ater* individuals ($F_{(7,78)} = 0.15$, $P > 0.05$). This indicates that both males and females were caught in equal amounts regardless of treatment (Figure 6.6).

Figure 6.5 Mean number of male and female *H. ater* individuals caught in each treatment.



A= α -pinene, AE= α -pinene and ethanol, B= β -pinene, BE= β -pinene and ethanol, C= control, E= ethanol, T= raw turpentine, TE= raw turpentine and ethanol.

6.4 Discussion

The results show that *H. ater* and *H. ligniperda* are attracted to host volatiles. This is consistent with other species of secondary and non-aggressive bark beetles (Heikkinen 1977, Hines & Heikkinen 1977, Norlander *et al* 1986, Löttyniemi *et al* 1988b, Byers 1989, Eidmann & Lindelöw 1991, Tunset *et al* 1993, Wilson & Day 1995, Flechtmann *et al* 1999). Primary attraction to host volatiles has been shown for a number of *Hylastes* species. *H. brunneus* and *H. cunicularis* can distinguish between different combinations of α -pinene and ethanol (Schroeder & Lindelöw 1989, Lindelöw 1992). *H. nigrinus* responds to α -pinene and/or ethanol (Witcosky *et al* 1987), β -pinene and other components of Douglas-fir resin (including Douglas-fir resin) (Rudinsky & Zethner-Møller 1967). *Hylastes opacus* is attracted to α -pinene (Schroeder & Lindelöw, 1989).

The results show that *H. ater* and *H. ligniperda* were able to distinguish between host volatiles in flight, prior to landing and in the absence of any feeding behaviour. This is demonstrated by higher catches in some volatile treatments compared to the control treatment. While other mechanisms may aid in host selection (e.g. visual cues, Eidmann *et al* 1991, Raffa *et al* 1993), these were not investigated in this study. However, these two species appear to be primarily orientating toward host volatiles. The mechanisms of host selection are not fully known for bark beetles, but volatiles have been shown to be the most powerful orientation cues (Delorme & Payne 1990, Eidmann *et al* 1991, Byers 1992, Tunset *et al* 1993).

H. ligniperda was attracted to the monoterpene lures and ethanol. Orientation to these attractants by *H. ligniperda* is consistent with many studies (Witcosky *et al* 1987, Byers 1989, Lindelöw 1992, Tunset *et al* 1993). The enhanced attraction to the monoterpenes and raw turpentine with ethanol over any volatiles alone demonstrates the strong synergistic effect of adding ethanol to the volatiles. Synergism between ethanol and volatiles has been well documented (e.g. Moeck 1970, Schroeder 1988, Phillips *et al* 1988, Schroeder & Lindelöw 1989, Phillips 1990, Rieske & Raffa 1990, 1999, Byers 1992, 1995, Jacobi 1992, Lindelöw *et al* 1993). Chénier & Philogène (1989) report that species of bark beetle attracted to raw turpentine from host species show enhanced attraction with the addition of ethanol. Combining α -pinene and ethanol, and a terpene blend and ethanol has a synergistic effect of enhancing attraction by *Hylobius abietis* (Tilles *et al* 1986a,b, Rieske

& Raffa 1990, 1999). *Hylastes cunicularis*, *H. brunneus* and *H. salebrosus* Eichhoff also demonstrate greater attraction to α -pinene and ethanol than to either component alone (Schroeder & Lindelöw 1989, Phillips 1990).

The role of ethanol in enhancing attraction is often attributed to ethanol production in hosts. Ethanol is produced when plant material is decomposed (Cade *et al* 1970, Moeck 1970, Schroeder 1988, Byers 1992, Lindelöw *et al* 1993). For many species of secondary or non-aggressive bark beetles that orientate toward dead or weakened hosts, the presence of ethanol with host terpenes represents the suitability of a host for colonisation (Byers 1992). Schroeder & Lindelöw (1989) observed enhanced attraction by *Hylastes cunicularius* and *H. Brunneus* to α -pinene and ethanol. Attraction differed with varying rates of ethanol release reflecting differences in breeding substrate (Schroeder & Lindelöw 1989). *Hylurgops palliatus* displayed enhanced attraction to α -pinene and ethanol, with the amount of ethanol being related to the age of host decay (Schroeder 1988).

In this study *H. ater* showed greatest attraction to volatiles with the addition of ethanol. The addition of ethanol to both raw turpentine and β -pinene greatly enhanced attraction compared with the volatiles on their own. The results confirm results from other studies regarding the synergistic effects of ethanol. However, *H. ater* did not show an attraction to the α -pinene lure, either in combination with ethanol or alone. The α -pinene treatments performed no better than the control.

H. ater is attracted to low concentrations of α -pinene and repelled at higher concentrations (Perttunen 1957, Rudinsky & Zethner-Møller 1967). *Hylastes cunicularius* is attracted to logs and turpentine but not attracted to α -pinene, the predominant volatile in many conifers (Eidmann *et al* 1991, Lindelöw *et al* 1993). The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, is attracted to α -pinene, but repelled by β -pinene (Heikkinen & Hrutfiord 1965). Douglas-fir (the host of *D. pseudotsugae*) has low amounts of β -pinene compared with levels of α -pinene. Heikkinen & Hrutfiord (1965), suggest *D. pseudotsugae* usually does not attack the crown and wounds of mature trees and the bark of young trees, all of which have higher levels of β -pinene. A survey of variation and inheritance of pinene composition in New Zealand *P. radiata* oleoresin reported that β -pinene was the dominant terpene (Burdon *et al* 1992a). While the relative amounts of the

terpenes varied with tree age and genetic background, the monoterpene fraction of samples was usually greater than 95% and β -pinene was almost always predominant, and accounted for on average, 70-80% of the α -pinene and β -pinene ratio from different populations (Burdon *et al* 1992a).

Burdon *et al* (1992a) indicate that as *P. radiata* ages the concentration of β -pinene increases. The stumps of mature trees may be more attractive to *H. ater*. *Hylastes* species associated with ponderosa pine are attracted to raw oleoresin alone or in combination with fermented phloem (Vité and Gara 1962, Löttyniemi *et al* 1988b, Phillips 1990). The enhanced attraction to the raw turpentine and ethanol mix over β -pinene and ethanol indicates the other components of oleoresin (present in the raw turpentine) were also important to the orientation of *H. ater* to the host.

Neither, *H. ater* or *H. ligniperda* were attracted to raw turpentine in the absence of ethanol. Used as an attractant (refer to Chapter Two) to investigate the flight activity of *H. ater* and *H. ligniperda*, raw turpentine was not effective. The results show that β -pinene and ethanol would have been a more effective treatment to catch greater numbers of the two species. However, while the raw turpentine treatment was not attractive, the numbers caught were an accurate representation of the relative activity or abundance of the two species. While other treatments were more attractive overall, the relative attractiveness was different between *H. ater* and *H. ligniperda*. For example, β -pinene and ethanol was 27 times (on average) more attractive than the control to *H. ligniperda*, whereas the same treatment was only 3.5 times (on average) more attractive to *H. ater* than the control.

While β -pinene is the dominant terpene in *P. radiata*, α -pinene is also present in reasonable quantities (Burdon *et al* 1992b). There was no explanation as to why *H. ater* was attracted to one monoterpene and not the other in this study. An attraction to β -pinene mixes over α -pinene mixes can be explained in terms of the dominance of terpenes in the oleoresin, however this does not explain the failure of α -pinene to attract *H. ater*. One possible explanation may be that the concentration of α -pinene being released from the lures was too strong (Perttunen 1957, Rudinsky & Zethner-Møller 1967). Another possible explanation is that the chirality of the α -pinene and β -pinene lures used in this study was not known. *Dendroctonus valens* has been reported to respond differently to the (R) (+)

and (S) (+) enantiomers of α -pinene (Hobson *et al* 1993, White & Hobson 1993). *H. ater* may respond in a similar way to different enantiomers of α -pinene.

While the attraction by *H. ater* to volatiles appeared to be more specific than *H. ligniperda*, both species were strongly attracted to β -pinene and ethanol, and raw turpentine and ethanol. The results indicate that if both species are to be caught then either combination of these compounds is recommended. Although the raw turpentine and ethanol mix does not attract *H. ligniperda* as well as β -pinene and ethanol, this combination is effective considering the lesser cost.

H. ater shows a clear preference toward host volatiles when combined with ethanol. Furthermore, the results show that both sexes were attracted to volatiles in equal numbers. Both sexes were present in the site in equal numbers. Monogamy in *H. ater* has been proposed and discussed by Crowhurst (1969) and Clark (1932b). Monogamy has also been suggested for other species of *Hylastes* (Kirkendall 1983, Tunset *et al* 1993, Kirkendall *et al* 1997).

The arrival of both male and females together to host material has been suggested as a mechanism to facilitate mate finding and gallery establishment (Tunset *et al* 1993). Tunset *et al* (1993) suggest that small differences in the sex ratio of colonising *Hylastes brunneus* adults, indicates orientation to host volatiles, rather than pheromones. Similar observations have been made for other bark beetle species (Rudinsky & Zethner-Møller 1967, Tilles *et al* 1986a,b, Löttyniemi *et al* 1988a, Eidmann *et al* 1991, Zagatti *et al* 1997). Species that require the use of pheromones to overwhelm hosts are usually colonised by one sex first (usually the female) (Alcock 1982, Raffa *et al* 1993).

H. ater (and most species of *Hylastes*) does not colonise living host material. Therefore, there is no requirement for the aggregation of large numbers of individuals to overwhelm potential hosts. As both sexes are attracted to volatiles in equal numbers, it is unlikely that an attraction pheromone is required for mate finding (Tunset *et al* 1993). The results also indicate that *H. ater* is unlikely to use pheromones for either aggregation or mate finding. This is consistent with findings from other species with similar host colonisation strategies (Wood, D.L. 1982, Raffa *et al* 1993). Aggregation by *H. ater* feeding in host material has

been observed (Milligan 1978, personal observations). This may be due to large numbers of beetles present in an area at the time. *H. ater* may be attracted to host material where other beetles are feeding. Similar observations have been made for *Hylobius abietis* (Tilles *et al* 1986a, Zagatti *et al* 1997). Tilles *et al* (1986a) suggest that this was not evidence of an aggregation pheromone, but was likely due to increases in the release of ethanol resulting from feeding.

The results show that raw turpentine and ethanol was an effective and low cost attractant to *H. ater* and *H. ligniperda*. Both species respond strongly to host volatiles in combination with ethanol and use these as the primary means of host orientation and selection. *H. ater* individuals are unlikely to utilise sex or aggregation pheromones in host colonisation or mating behaviour.

7. GENERAL DISCUSSION

7.1 Introduction

The extent of attack of *P. radiata* seedlings in New Zealand second rotation forests by *H. ater* was evaluated. Prior to this research there was considerable confusion with regard to the pest status of *H. ater* in New Zealand. This resulted from workers failing to identify the dominant cause of seedling mortality associated with *H. ater* attack, or by misdiagnosing *H. ater* mortality. Misdiagnosis of seedling mortality by bark beetle and weevil attack is commonly reported in other studies (e.g. Tribe 1991b). Beetles often leave before damage is noticed and damage is often below the ground (Du Toit 1975, Tribe 1990, 1991b, Rieske & Raffa 1991, Eidmann 1992, Lindelöw 1992, Atkinson & Govender 1997). In contrast the bark weevil *H. abietis* attacks above ground and mortality due to this damage is obvious (Lindelöw 1992, Leather *et al* 1999).

Historically, studies of *H. ater* in New Zealand focused seedling mortality resulting from *H. ater* attack. The death of seedlings is of primary importance to the establishment of second rotation forests. As well as investigating mortality, this research examined the effects of sub-lethal attack. Understanding the impact of seedling attack means more accurate decisions may be made with regard to the management of *H. ater*. By investigating aspects of the ecology and behaviour of *H. ater* this research has provided valuable information that may be incorporated into the management of *H. ater*. This information is in two areas i) being able to predict areas that may be at high risk to *H. ater* attack, and ii) developing strategies to minimise impacts by *H. ater*.

One problem with investigating *H. ater* is the relative lack of literature describing the impact of the species in other areas. Other *Hylastes* species (e.g. *H. angustatus* (Du Toit 1975, Tribe 1990, 1991b), *H. brunneus* (Löyttyniemi *et al* 1988b), *H. nigrinus* (Bedard *et al* 1990, Jacobi 1992, Witcosky *et al* 1986a,b), *H. cunicularis* (Eidmann 1992, Lindelöw 1992, Atkinson & Govender 1997), *H. porculus* (Klepzig *et al* 1996b)) attack pines and other species (e.g. spruce and Douglas-fir) with similar levels of mortality as reported in New Zealand. However, the biology of *Hylastes* species and the associated damage are still poorly understood (Zethner-Møller & Rudinsky 1967, Löyttyniemi *et al* 1988b).

H. ater is regarded as a pest of pine seedlings in Britain and Europe. However its status is lessened due to the role of the more aggressive weevil *Hylobius abietis* (Zethner-Møller & Rudinsky 1967, Heritage *et al* 1989, Lindelow 1992, Wilson & Day 1995). *H. abietis* is regarded as the most important pest of conifer plantations in Britain and Europe (Heritage *et al* 1989, Norlander 1989, Lindgren *et al* 1996, Manlove *et al* 1997, Hagner & Jonsson 1995, Salisbury & Leather 1998, Leather *et al* 1999, Örlander & Nilsson 1999). Control measures have been estimated to cost approximately \$NZ12M per annum in Britain (Heritage *et al* 1989). Congeneric species *H. pales* fills a similar niche in North America (Nord *et al* 1984, Klepzig *et al* 1991, Nevill & Alexander 1992, Salom 1997, Rieske & Raffa 1999, Rieske 2000) and is often associated with *Pachylobius* and other weevil species (*picivorus* (Franklin & Taylor 1970, Klepzig *et al* 1991). These species cause substantial damage to Christmas tree plantations (Lewis & Alexander 1986, Rieske 2000). While these weevils belong to a different taxonomic group than *Hylastes* species, they occupy similar niches and demonstrate similar feeding and breeding behaviours.

In New Zealand *H. ater* attacks on seedlings may be evaluated in the absence of the more aggressive species associated with *H. ater* in other countries. Bark beetles and weevils which attack seedlings are often part of a complex of species occupying similar habitats and causing similar damage to seedlings (Zethner-Møller & Rudinsky 1967, Franklin & Taylor 1970, Witcosky *et al* 1986a,b, Löyttyniemi *et al* 1988b, Bedard *et al* 1990, Klepzig *et al* 1991, Jacobi 1992, Lindelow 1992, Wilson & Day 1995, Leather *et al* 1999). Research efforts have often focused on the most aggressive or destructive species, and the influence of individual species may be difficult to determine (Tribe 1990, 1991a,b, Lindelow 1992)

Hylurgus ligniperda occupies a similar niche to *H. ater* in New Zealand. The interactions between *H. ater* and *H. ligniperda* are important to forestry. These two species compete for breeding substrate, rather than as a species complex attacking trees. While Ciesla (1988) reports that *H. ligniperda* attacks *P. radiata* seedlings in Chile, there is no evidence to suggest that *H. ligniperda* attacks seedlings in New Zealand (Bain 1977, personal observations) or South Africa (Tribe 1991a,b). This study will be valuable to those investigating a complex array of species that attack seedlings. Seedling attacks by *H. ater* in other countries may be more significant than are currently reported.

In New Zealand *H. ater* and *H. ligniperda* have significant interactions (Chapter Two). It was not possible to fully investigate the nature of this relationship, beyond documenting the dominance of each species at different times of the year. *H. ligniperda* has a competitive advantage over *H. ater* during the majority of the summer period. The same is true in Chile (Ciesla 1988). Competitive interactions between these species are an important topic for future research. Observations by Clark (1932b) and Crowhurst (1969) prior to the introduction of *H. ligniperda* indicate that the introduction of *H. ligniperda* has limited the abundance of *H. ater* during the summer period. The number of sites where seedlings may be at risk to *H. ater* attack has decreased as a result.

H. ater is the dominant cause of mortality in the first year of establishment (Chapter Three). While seedling mortality above 15% is not common in New Zealand, this may in part, be due to current operation practices (and will be discussed further below). However, in sites harvested at the end of summer, seedling mortality resulting from *H. ater* attack is often higher. Historically, mortality as high as 50% has been observed (Zondag 1964, 1965) and may reflect operational practices at the time. Planting high-risk sites following colonisation by *H. ater* is not currently common practice, but this is due to other factors rather than an attempt to minimise impacts by *H. ater*. Currently companies tend to delay the planting of high-risk sites (harvested at the end of summer) until the winter of the following year. Any change in current operational practices may effect the number of seedlings at risk from *H. ater* attack. For example, planting during winter immediately following the harvesting of high-risk sites would expose a greater number of seedlings to attack by *H. ater*. Practices such as planting container grown seedlings (currently it is common practice to plant bare rooted seedlings) would minimise the planting stresses seedlings are currently subjected too. This would mean the planting season could be extended (S. Downs, personal communication) putting more seedlings at risk of attack.

Most research investigating attack by bark beetles and weevils has focused on seedling death (Du Toit 1975, Norlander 1989, Tribe 1990, 1991b, Eidmann 1992, Jacobi 1992, Lindelöw 1992, Hagner & Jonsson 1995, Lindgren *et al* 1996, Atkinson & Govender 1997, Manlove *et al* 1997, Salisbury & Leather 1998, Leather *et al* 1999). In addition to determining the level of mortality, the frequency and severity of sub-lethal attack were investigated in this study. Seedlings commonly recovered from severe attack. Sub-lethal attack did not effect the growth of seedlings in the first two years following planting.

While the influence of attack on seedling growth has not been investigated in many other studies, Leather *et al* (1999) suggest the effects of sub-lethal attack may not be apparent until some time following attack. Örlander & Nilsson (1999) observed growth losses in *Picea abies* seedlings sub-lethally attacked by *Hylobius abietis* one year after planting. The trials in this study will need to be re-measured in the future to confirm the results. If sub-lethal attack by *H. ater* does effect seedling growth the pest status of *H. ater* will have to be re-addressed. For example, if attacked seedlings are shorter at thinning (5-7 years) then the practice of selecting seedlings on form characteristics may mean greater numbers of seedlings are compromised at thinning.

Seedlings that are severely attacked by *H. ater* are more likely to be invaded by sapstain fungi (Chapter Four). Fungi are commonly associated with more aggressive tree killing bark beetles (Klepzig *et al* 1991, Raffa 1991, Paine *et al* 1997). The spread of root diseases such as *Leptographium procerum* and *L. wagnerii* are associated with bark beetle activity (including species of *Hylastes*) (Harrington *et al* 1985, Witcosky & Hansen 1985, Witcosky *et al* 1986a,b, Jacobi 1992, Klepzig *et al* 1996a). More recently, *H. abietis* has been identified as a vector of *L. procerum* and other species of fungi (Lévieux *et al* 1994) and may vector these fungi to seedlings during attacks (Leather *et al* 1999). *H. pales*, *Pachylobius picivorus* and other bark weevils have been implicated as vectors of *L. procerum* to *Pinus* species in North America (Wingfield 1983, Lewis & Alexander 1986, Witcosky *et al* 1986a, Klepzig *et al* 1991, Nevill & Alexander 1992, Salom 1997). Nevill and Alexander (1992) demonstrated the ability of *H. pales* and *Pissodes nemorensis* to transmit *L. procerum* to eastern white pine seedlings (*Pinus strobus* L.).

This study demonstrates for the first time a relationship between sub-lethal *H. ater* attack and the subsequent invasion of seedlings by sapstain fungi in New Zealand. While seedling invasion by sapstain is not fully understood, there is potential for this relationship to be very significant to the forest industry. This is a new area of research and I think it is the most significant finding in this study. While it is difficult to determine the economic impact, from an applied entomology perspective, the relationship between *H. ater* attack and subsequent invasion of seedlings by sapstain fungi is very important. It may be possible to determine the full effect of seedling invasion by sapstain fungi and the cost to the forest industry that results from timber staining or by fungi having a pathogenic effect on seedlings. One of the most significant consequences of this relationship is the potential

for *H. ater* to vector new diseases or fungi that may be introduced into New Zealand in the future. Due to the association of *H. ater* with fungi, it may also be appropriate to re-evaluate the status of *H. ater* (and *H. ligniperda*) as quarantine organisms. It is possible that new introductions of these species may carry fungi not currently established in New Zealand. *H. ater* may be able to vector root diseases or fungi such as pitch canker (*Fusarium subglutinans*) (Fox *et al* 1991, Dick & Bain 1996, Dick 1998). It is important that the pest status of *H. ater* is considered to reflect the potential association of the species with any new introduction of this type into New Zealand.

7.2 Options for the control of *H. ater* in New Zealand

A wide range of control options are available to protect seedlings from attack by bark beetles and weevils (e.g. see Leather *et al* 1999). Historically, control focused on the removal of habitat by burning and by trapping beetles and destroying them (Munro 1926, 1929, Forestry Commission 1946). Traps and trap logs are said to protect trees from attack by more aggressive bark beetles (i.e. *Ips typographus* L.) (Grégorie *et al* 1997, Niemeyer 1997). In Chapter Six volatiles were identified that attract large numbers of *H. ater*. However, the amount of harvesting activity in *P. radiata* forests means that trapping would be unable to reduce beetle numbers to a level where effective control would be economical (Löyttyniemi *et al* 1988a,b). The best performing attractants identified in Chapter Six may be used effectively in monitoring programmes or in future research that requires the trapping of large numbers *H. ater* (Norlander 1987, Norlander 1989, Zumr & Starý 1994, Rieske & Raffa 1999, Rieske 2000).

A variety of physical barriers (e.g. stockings, collars) have been proposed to protect seedlings from attack by *H. abietis*. While these have been shown to be effective they have not been widely used in forestry operations (Lindstrom *et al* 1986, Eidmann & von Sydow 1989, Hagner & Jonsson 1995, Eidmann *et al* 1996, Watson 1999). Site preparations such as ripping and stump removal have been reported to be effective in reducing *H. abietis* numbers (Scott & King 1974, von Sydow 1997, Örlander & Nilsson 1999). However, this would not be economical at the scale required in New Zealand. Leather *et al* (1999) summarise some of the natural enemies of *H. abietis* in Britain and Europe. Control by predators or parasitoids may contribute to reductions in *H. abietis*

populations in some situations, and may be useful in conjunction with other management techniques (Leather *et al* 1999). In New Zealand predatory Coleoptera larvae were isolated with *H. ater* larvae in stumps (Chapter Two). However these were seldom encountered. There was no evidence to suggest that they may reduce *H. ater* numbers. Past attempts have been made to introduce biological control organisms into New Zealand (Zondag 1976b, 1979, Zondag *et al* 1976, Faulds 1989). None of the species introduced for the purpose of control were ever recovered following release (Crowhurst 1969, Faulds 1989, personal observations). It is unlikely that support could be gained to investigate further biological options for the control of *H. ater* in New Zealand. Fungi and nematodes are known to infect larval and adult stages of *H. abietis* (Leather *et al* 1999). Nematodes are reported to reduce *H. abietis* numbers under certain conditions in the field (Leather *et al* 1999). Wood colonising fungi have been suggested to reduce habitat quality and stump attractiveness to colonising weevils (Skrzecz & Moore 1997). While both nematodes (Dale 1967) and fungi (Chapter Four) are associated with *H. ater* in New Zealand, there is no evidence to suggest that either is capable of controlling beetle populations. Feeding deterrents (e.g. verbenone) suppress *H. abietis* feeding activity (Salom *et al* 1994, Zúmr & Starý 1995, Lindgren *et al* 1996, Salom *et al* 1996, Klepzig & Schlyter 1999, Watson 1999) and may be of use in future bark beetle management programmes.

Currently the most effective method of protecting seedlings from bark beetle and weevil attack is by using pre- and post- planting insecticides (Nord *et al* 1984, Norlander 1989, Heritage 1997a,b, Örlander & Nilsson 1999). Pre-planting treatments involve dipping bare rooted seedlings in a high concentration of insecticide (Stoakley & Heritage 1989, 1990a,b). Post-planting treatments may be applied by spraying seedlings following planting (Heritage *et al* 1997b, Heritage & Johnson 1997) although these may not give acceptable levels of protection (Heritage *et al* 1989). Marshal suSCon[®] is a slow release granular insecticide. The active ingredient is a systemic carbamate insecticide (carbosulfan) (Lemperière & Julien 1989). The treatment gives protection from bark beetles and weevils for 20 months, although seedlings are unprotected for a short time until the insecticide is taken up into the seedling (Mrlina *et al* 1994). In addition, weevils must sample bark before they are deterred (Mrlina *et al* 1994, Heritage *et al* 1997a). Leather *et al* (1999) suggest an ideal insecticide for use against *H. abietis* would be systemic and mask or modify the host volatiles which make seedlings attractive.

The efficacy of Marshal suSCon[®] to protect *P. radiata* seedlings from attack by *H. ater* was tested in Chapter Three. As well as providing protection to seedlings by reducing the severity of attack, Marshal suSCon[®] appears to act as a feeding deterrent to *H. ater*. *H. ater* seldom attacks seedlings above the ground, unlike *H. abietis* (Leather *et al* 1999), and is likely to encounter the insecticide granules in the soil. Therefore, treated seedlings are less likely to be attacked and the effects associated with sub-lethal attack (e.g. invasion by sapstain fungi) may be minimised.

One problem of using prophylactic treatments is that the timing of application is crucial (Tribe 1991b). Many studies document the use or development of attractants to monitor and forecast beetle and weevil activity for the purpose of insecticide application (Norlander 1987, Löyttyniemi *et al* 1988b, Norlander 1989, Rieske & Raffa 1990, Tribe 1990, Zumr & Starý 1994, Rieske & Raffa 1999, Rieske 2000). In order for prophylactic treatments to be effective they must be applied prior to attack (Tribe 1991b, Leather *et al* 1999). *H. abietis* can attack seedlings soon after they are planted (Leather *et al* 1999).

The timing of the application of Marshal suSCon[®] is not such a relevant issue in New Zealand. While the systemic action may take a week or so to provide protection throughout seedlings (Mrlina *et al* 1994) this is not a significant factor when protecting seedlings from *H. ater* attack in New Zealand. The treatment is applied during planting (winter). As *H. ater* individuals do not emerge until mid to late summer there is ample time for seedlings to be protected. Therefore, Marshal suSCon[®] is an effective treatment to protect seedlings from *H. ater* attack in New Zealand.

One of the most effective control strategies for bark beetles and weevils is to leave land fallow (delay planting) for a sufficient period of time to allow beetles and weevils to depart from the area. Delayed planting was the prescribed method of control before chemical and trapping methods were available (Munro 1929, 1927, Clark 1932b, Lindelöw 1992, Leather *et al* 1999) and is still an effective control method for many species (e.g. *Hylastes cunicularis*, *H. brunneus*) (Scott & King 1974, Norlander 1989, Tribe 1990, Lindelöw 1992). However, as species of *Hylobius* and other bark beetle species may remain in an area for considerable periods of time (commonly 2-3 years, and up to 5 years) (Franklin & Taylor 1970, Scott & King 1974, Nord *et al* 1984, Nordenhem 1989, Tribe 1990, Hagner & Jonsson 1995, Örlander & Nilsson 1999, Rieske & Raffa 1999), the costs associated

with leaving areas fallow may mean this technique is not economically practical (Nord *et al* 1984, Ciesla 1988). In addition, adult *Hylobius* species will often migrate into planting areas from the surrounding landscape and cause considerable damage (Lindelöw *et al* 1993, Zúmr & Starý 1994, Leather *et al* 1995).

In New Zealand, *P. radiata* stumps usually remain suitable for *H. ater* colonisation until the first generation of beetles has emerged (Chapter Two and Three). The emergence of the first generation of beetles will degrade stumps to the point where they are not attractive to colonising beetles. In comparison, stumps in Europe may be suitable breeding sites for longer periods (Nordenhem 1989). During summer months in New Zealand, stumps may only be a suitable breeding habitat for two to three months (Chapter Two). For sites harvested prior to winter, generation times are a lot longer (up to 13 months). In New Zealand planting is undertaken during the winter period. Due to interactions between *H. ater* and *H. ligniperda* it is only necessary to delay planting in sites harvested after mid-summer. In these cases planting will only need to be delayed till the following winter, not for several seasons as required for seedlings vulnerable to attack *Hylobius* species (Franklin & Taylor 1970, Scott & King 1974, Nord *et al* 1984, Hagner & Jonsson 1995, Leather *et al* 1999, Rieske & Raffa 1999). The suitability of delayed planting as a management technique will be discussed below.

7.3 Site-risk assessment and damage forecasting

Predicting sites where seedlings are most likely to be attacked by bark beetles is a useful management tool (Wilson *et al* 1996, Leather *et al* 1999). Predicting the likelihood and time of attack provides useful information when determining the best time for the application of insecticide treatments (Norlander 1987, Löyttyniemi *et al* 1988b, Norlander 1989, Rieske & Raffa 1990, Tribe 1990, Zúmr & Starý 1994, Rieske & Raffa 1999, Rieske 2000.). Advance knowledge of the potential risk to a site means the most effective control strategy may be implemented (Leather *et al* 1999). The terminology ‘hazard’ represents those factors that may predispose a seedling to attack, while ‘risk’ is used to describe the abundance of the insect (Leather *et al* 1999). Determining the effects of hazards (i.e. aspect, slope etc.) was beyond the scope of this study. Leather *et al* (1999) suggest that it is difficult to separate the effects of ‘risks’ from ‘hazards’ when determining the extent to

which they may influence the amount *H. abietis* attack on seedlings. It was more appropriate to assess the risks (identify areas most likely to have high beetle populations) associated with plantings in this study.

Leather *et al* (1999) review the risk factors that may influence *H. abietis* damage on conifers. Four main groups of factors are suggested to influence *H. abietis* populations i) the suitability of breeding sites ii) weevil development rate iii) planting site factors and iv) weevil-seedling interactions (Leather *et al* 1999). Many studies have attempted to forecast damage by *H. abietis* and a number of risk prediction models have been proposed, with a few demonstrating limited successes (Leather *et al* 1999). Wilson *et al* (1996) suggest four variables were significant predictors of damage by *H. abietis* in a recent study which identified 45 potential variables. The four variables were: the size of the planted area, the age of the planting, whether seedlings were self-seeded or planted and whether a site was newly planted or previously harvested. The age of the clear-fell area was also a dominant factor recognised by Örlander and Nilsson (1999) who also found the planting of seedlings on mounds reduced attack.

A number of aspects of the biology and behaviour of *H. abietis* makes risk prediction more difficult compared to those associated with *H. ater* in New Zealand. Firstly, sites in New Zealand are able to sustain only one generation of *H. ater* (or *H. ligniperda*) before breeding material is exhausted. In other areas, breeding material for weevils and bark beetles may remain suitable for longer periods (Franklin & Taylor 1970, Scott & King 1974, Nordenhem 1989, Nord *et al* 1984, Lindelöw 1992, Hagner & Jonsson 1995, Örlander *et al* 1997, Rieske & Raffa 1999, Örlander & Nilsson 1999). *H. ligniperda* is the dominant species of the pair in the majority of sites in New Zealand. This means a substantial amount of area that is harvested each year is less suitable for *H. ater* breeding.

The second important difference between *H. ater* and species of *Hylobius* is that *Hylobius* individuals migrate into planted sites from other sites (Lindelöw *et al* 1993, Zúmr & Starý 1994, Leather *et al* 1995, Manlove *et al* 1997). This migration is difficult to predict (Zúmr & Starý 1994, Manlove *et al* 1997, Leather *et al* 1999). Weevils may walk or fly up to distances in excess of 100 km into recently planted sites from surrounding areas (Solbreck 1980, Nilssen 1984, Zúmr & Starý 1994, Leather *et al* 1995). However, Leather *et al*

(1995) suggest flights of large distances are often not necessary as harvesting and planting activities are seldom far from each other.

In New Zealand, *P. radiata* stumps are usually degraded following one generation of beetles and appear unattractive to colonising beetles. While there may be *H. ater* flight activity in older sites, seedlings are seldom attacked by beetles. This was most evident in this study when one area of a site was harvested at the beginning of summer, while the remaining area was harvested at the end of summer. Seedlings in the high-risk areas harvested at the end of summer were frequently attacked. Seedlings planted in the low-risk (older) areas were not attacked. On occasions, one-year old seedlings planted in high-risk sites were frequently attacked, while one-year old seedlings planted in adjacent low-risk sites were not attacked. *H. ater* appears to only be able to detect seedlings planted directly amongst emerging beetles. Adults that do not emerge among seedlings migrate (by flight) to areas containing breeding or food material. Harvesting activities in New Zealand *P. radiata* forests are continuous all year round. Volatiles are emitted from recently harvested sites. Migrating beetles should easily detect these volatiles and colonise these sites. Adult beetles do not appear to detect seedlings while flying. The flight activity of adults in older sites is in response to volatiles from recently harvested sites. Harvesting activities are usually in close proximity (less than 10 km).

These differences between *H. ater* and *H. abietis* mean it is possible to make simple but effective predictions of risk. Sites harvested toward the end of summer (February-March) are the most likely to be high-risk sites. As the period between harvesting and planting increases, the risk that seedlings will be attacked by *H. ater* decreases. While a few seedlings in sites harvested between October and February were attacked by *H. ater* (Chapter Three) the frequency and severity of these attacks were considerably less than other sites. Attack in sites planted prior to October (approximately nine months before planting) was minimal. If sites harvested just prior to April (when *H. ater* was the most abundant) were planted during the following winter (July), the likelihood of frequent and severe attacks would be very high (Chapter Three).

While the 'factors' (or 'hazards') described by Leather *et al* (1999) would effect the severity of *H. ater* attacks, these could not be assessed during this study. Site factors may influence attack dynamics in two ways: Firstly, both environmental and biological factors

may influence *H. ater* population dynamics and larval survival, affecting the population of beetles emerging in a site. Secondly, certain site factors may influence the ability of a seedling to respond to attack. Separating the relative influence of all contributing factors is very difficult due to potential interactions between them (Leather *et al* 1999). In addition, any attempt to investigate these interactions may be complicated by genetic variation of resistance traits for seedlings planted in different sites.

A simple risk-prediction system based on harvesting history (as described above) is able to provide forest managers with enough information to make the most appropriate and cost-effective management and control plans prior to planting. High-risk sites are those harvested between February and April and planted the following winter. Seedlings planted in these sites must be protected from *H. ater* attack. Moderate-risk sites are those harvested between October and January. The treatment of seedlings in these sites is advisable, but the associated costs would need to be considered in terms of the potential benefits gained from treatment. Finally, low risk sites are those harvested prior to October (harvested during winter months) and planted the following winter. It is unlikely that any treatment would be cost-effective in these sites given the low risk of attack.

7.4 The development of a management strategy to minimise risks to seedlings associated with *H. ater* attack

In operational forestry, a management strategy to protect seedlings from *H. ater* attack should be able to provide protection while being cost-effective. Determining the costs of *H. ater* damage and alternative forms of control may appear simple, but is more complicated.

Determining the economic threshold for seedling mortality for any given treatment is relatively simple. Viability of treatment needs consideration of the following cost per hectare of:

- The cost of any seedlings killed
- The potential cost of replanting these seedlings if necessary
- In cases of severe mortality, the cost associated with complete restocking
- The desired stocking required for the success of subsequent forestry operations

- The cost per seedling for any treatment. If this is an insecticide treatment it should include any additional costs associated with application. If land is to be left fallow (planting delayed until the following winter) this cost is approximately equivalent to the discounted value of one year's growth (Nord *et al* 1984) and any after-costs associated with returning the site to condition suitable for planting (i.e. weed control).

Determining the costs associated with sub-lethal attack is more difficult. While sub-lethal attack by *H. ater* does not effect seedling growth in the first two years after planting, growth losses may become apparent after longer periods (Leather *et al* 1999). Determining the value of growth losses due to *H. ater* attack is difficult, and may be confounded by other factors. Attacked seedlings are more likely to be invaded by sapstain fungi, but as the effects this are not fully understood, it is not possible to determine the cost of this. However, there is the potential for the invasion of seedlings by sapstain fungi to have significant impacts on the forest industry. Furthermore, the potential for *H. ater* to vector other pathogens or diseases to seedlings during sub-lethal attack means that the costs associated with attack may be greater if pathogenic fungi or diseases become established in New Zealand.

In most cases where sites are either high- or low-risk, determining the level or extent of treatment should be relatively simple (Figure 7.1). In the case of sites that are considered to be low-risk, treatment is not likely to be considered. For high-risk sites, it is most likely that management decisions will be either to delay planting until the following season or protect seedlings using prophylactic treatments (i.e. Marshal suSCon[®]). The cost of prophylactic treatment will probably be low compared with the costs associated with seedling mortality in high-risk sites, particularly if sites may require re-stocking. Current forestry practice is to lower initial stocking rates to the lowest levels without compromising the form and growth of seedlings. To be successful, this practice relies on high establishment rate in the initial planting. However, these types of management decisions are not driven by forest health concerns. Consequently, if high-risk sites are planted in the absence of protective treatments most of these sites will require some sort of 'blanking' (filling in) or replanting following *H. ater* attack.

The alternative to treating seedlings during planting in high-risk sites is to leave the land fallow until the following planting season. If the area of forest to be replanted in any year

is less than the area that was harvested, then it may be beneficial to plant those areas least at risk to *H. ater* attack first (assuming other management regimes do not dictate which areas are planted). Therefore, it will be possible to minimise the amount of high-risk areas to be planted. However, the cost of delaying planting in high-risk areas (e.g. weed control, the cost of leaving land fallow) may be less than the cost of alternative treatments.

It may be more difficult to determine the most appropriate treatment for sites that are characterised as being moderate-risk. In these cases it may be more economical to avoid treatment and hope that the cost of attack is less than the cost of treatment for these sites. This may be difficult when it is not possible to assess the cost of sub-lethal attack. In these cases visual inspections of larval populations may provide an indication as to the risk associated with planting in a site.

If seedling types can be identified that display resistance to *H. ater* attack and show satisfactory growth and form characteristics (Chapter Five), it may be beneficial to plant these in moderate-risk sites. Older, larger seedlings have been found to be more resistant to attack by *Hylobius abietis* (Selander *et al* 1990, Selander 1993, Örlander & Nilsson 1999). However, in the absence of attack there are no losses due to treatment costs. For such a management strategy to be implemented, growth or form losses by planting seedlings with superior resistance would have to be minimal. Planting resistant seedlings is a valuable management strategy for use in high-risk areas. As resistant seedlings are attacked with less severity (Chapter Five), seedlings are more likely to survive attack, and the secondary effects of attack may be minimised (Chapter Four). The use of resistant hosts is an ideal way to control insect pests (Wiseman 1994). Several studies have indicated the potential of resistant hosts for use in forestry (e.g. Alfaro & Ying 1990, Sahota *et al* 1994, Lieutier *et al* 1997a,b, Tomlin & Borden 1997a,b).

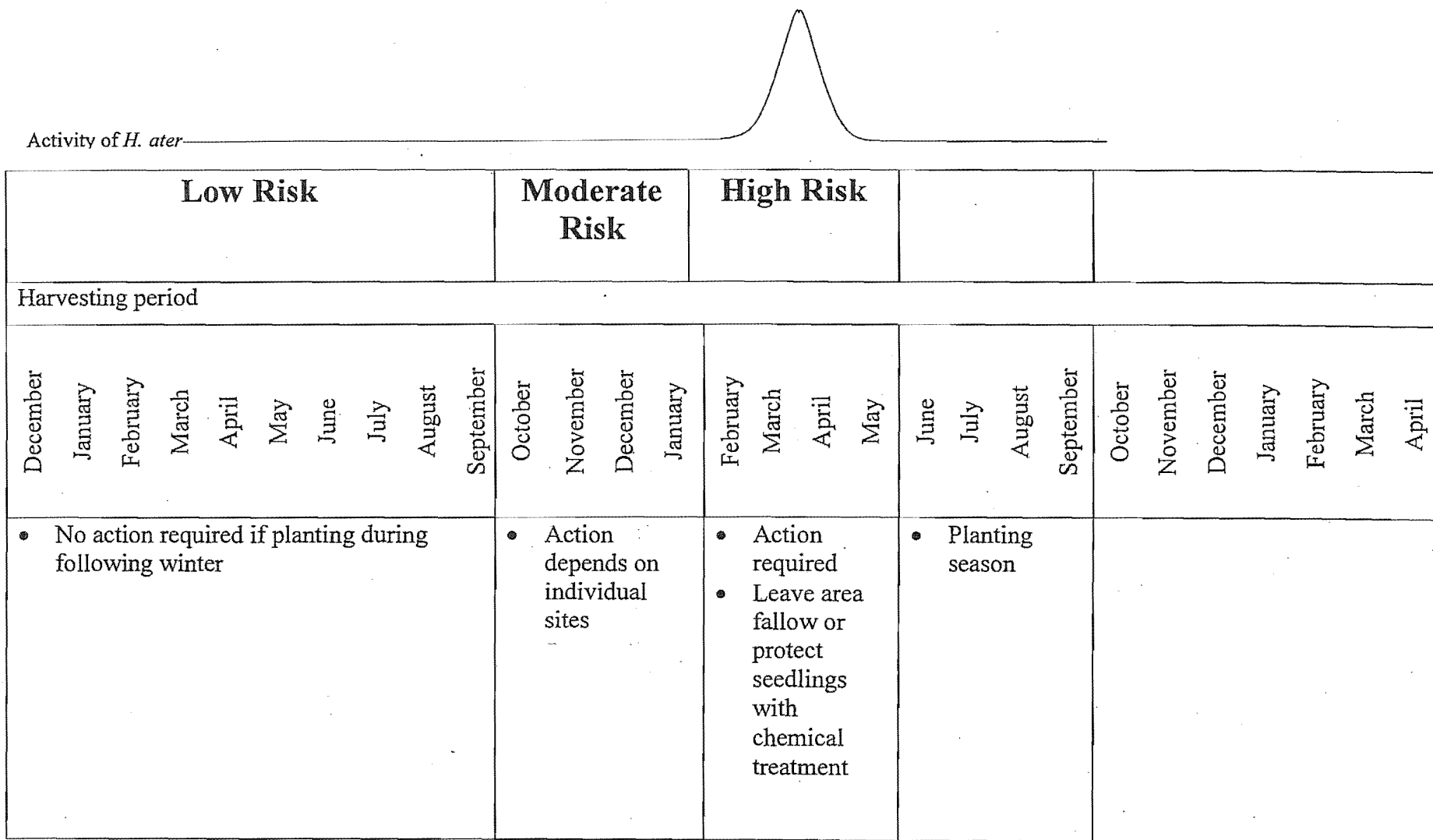


Figure 7.1 Determining the risks associated with *H. ater* attack.

7.5 The direction of *H. ater* research in New Zealand

H. ater is a significant pest in *P. radiata* forests in New Zealand. This thesis addressed many areas of *H. ater* research and made a substantial contribution to the understanding of this species. However further research is required to fully understand the ecology and behaviour of *H. ater* and to gain a better appreciation of its pest potential. The most pertinent research will investigate strategies that minimise impacts resulting from seedling attacks. Future research should include the following:

Ecology and behaviour of *H. ater*

Efforts should be made to determine the biological and environmental factors that influence *H. ater* populations. This will allow more accurate predictions to be made regarding 'high-risk' and 'high-hazard' areas. An understanding of the interactions between *H. ater* and *H. ligniperda* should be further developed.

Primary effects of *H. ater* attack

Sub-lethal attack did not influence the growth of seedlings in the first two years after planting. The effects of attack on growth may not be apparent until a greater time after planting. The potential effects of sub-lethal attack on growth should be evaluated when trees are more mature (i.e. 5-7 years).

Secondary effects of *H. ater* attack

There was a strong relationship between sub-lethal attack by *H. ater* and the subsequent invasion of seedlings by sapstain fungi. This is new research and has very important implications for the New Zealand forest industry. Consequently, much of what is understood is in its infancy and considerable work is required to properly understand the interactions between *H. ater* attack and the invasion of seedlings by fungi.

It is important to determine the dynamics of sapstain fungi following the invasion of seedlings. For example, it may be possible to quantify how long sapstain fungi remain in seedlings following *H. ater* attack by establishing some long-term monitoring studies on the fate of mature trees following attack. It is important to determine whether sapstain fungi continue to spread throughout seedlings following invasion, or are contained by subsequent tree growth.

The role of *H. ater* in vectoring fungi needs to be fully addressed. While a relationship between the invasion of seedlings by sapstain fungi and attack by *H. ater* has been established, the mechanism of invasion needs to be identified. In particular, are sapstain fungi vectored to seedlings by *H. ater* during attack, or do sapstain fungi invade *H. ater* wounds from the surrounding soil following attack?

Some seedling types are more resistant to *H. ater* attack. Investigating the response of different seedling types to invasion by sapstain fungi could indicate whether traits that enhance seedling resistance to *H. ater* attack also confer resistance to invasion by sapstain fungi. Alternatively, it may be possible to identify other factors that contribute to the ability of seedlings to resist fungal invasion.

Further surveys of sapstain species vectored by *H. ater* are required to determine those species of sapstain fungi that are associated with *H. ater*. This research will advance our understanding of the role of *H. ater* in the movement of fungi in forest systems. The effect of sapstain fungi on the health of seedlings will contribute to an awareness of the role of *H. ater* as a vector of other organisms, in particular pathogenic fungi.

Resistance of *P. radiata* seedlings to *H. ater* attack

Some seedlings are more resistant to attack by *H. ater* and the use of resistant genetic seedling material is an exciting direction in its management. Future research should investigate the effect of *H. ater* attack on the mortality of different seedling types. A greater genetic range of seedling material will need to be assessed to determine those that have traits that are most resistant to attack by *H. ater* while maintaining characteristics (growth and form) that are currently important. Investigating potential 'trade-offs' between resistance and growth is required to determine whether the benefits of using resistant material are profitable in operational forestry situations. Seedlings from different propagation techniques with the same genetic background should be assessed for resistance. If different propagation techniques confer superior resistance characteristics, then benefits may be immediately utilised. Finally, it is important to investigate whether seedlings that demonstrate superior resistance to *H. ater* also show superior resistance to other organisms (i.e. sapstain fungi).

Future research should investigate the behaviour of *H. ater* prior to seedling attack. For example, whether *H. ater* selects less resistant seedlings prior to attack. Determining whether *H. ater* is able to select stressed seedlings prior to attack will contribute to understanding of the affects of different management practices on the vulnerability of seedlings to attack.

Further study in all or any of the above areas will result in a greater understanding of the pest potential and management of *H. ater*. The understanding and management of forest health problems in New Zealand has been advanced through this research, as has our understanding of interactions of bark beetles with their hosts. Future research as outlined above will benefit forestry in New Zealand, as well make a substantial contribution to applied entomology at an international level.

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REFERENCES

- Abraham, L. D., Roth, A. Saddler, J. N. and Breuil, C. 1993. Growth, nutrition and proteolytic activity of the sap-staining fungus *Ophiostoma piceae*. *Canadian Journal of Botany* 71: 1224-1230
- Alcock, J. 1982. Natural selection and communication among bark beetles. *Florida Entomologist* 65: 17-32
- Alfaro, R. I. 1997. Current research on genetic resistance to white pine weevil in British Columbia. Pp 222-224 in Gregoire, J. C., Liebhold, A. M., Stephen, F. M., Day, K. R. and Salom, S. M. (eds) *Integrating cultural tactics into the management of bark beetle and reforestation pests*. General Technical Report NE-236. US Department of Agriculture, Forest Service, Northeastern Forest Experimental Station. Radnor, Pennsylvania USDA Forest Service.
- Alfaro, R. I. and Ying, C. C. 1990. Levels of Sitka spruce weevil, *Pissodes strobi* (Peck), damage among Sitka spruce provenances and families near Sayward, British Columbia. *Canadian Entomologist* 122: 607-615
- Alfaro, R. I., Kiss, G. K., King, J. and Yanchuk, A. 1996. Variation in the induced resin response of white spruce to attack by the white pine weevil. *Canadian Journal of Forest Research* 6: 967-972
- Alma, P. J. 1975. Problems of pest assessment: forestry pests. *New Zealand Entomologist* 6: 22-23
- Alma, P. J. and van Boven, R. J. 1976. Insect invasion and survival of Douglas-fir stumps in New Zealand. *New Zealand Journal of Forestry Science* 5: 306-312
- Amman, G. D. 1972. Mountain pine beetle brood production in relation to thickness of lodgepole pine phloem. *Journal of Economic Entomology* 65: 138-140
- Anderbrant, Ö. 1990. Gallery construction and oviposition of the bark beetle *Ips typographus* (Coleoptera: Scolytidae) at different breeding densities. *Environmental Entomology* 15: 1-8
- Atkins, M. D. 1966. Behavioural variation among scolytids in relation to their habitat. *Canadian Entomologist* 98: 285-288
- Atkinson, P. R. and Govender, P. 1997. Mortality of *Pinus patula* seedlings due to *Hylastes angustatus* (Herbst) (Coleoptera: Scolytidae). *Institute for Commercial Forestry Research Bulletin Series* 08/1997. South Africa. 15p.

- Bain, J. 1971. *Hylastes ater* attack on newly planted stock. Forest Research Institute Unpublished Report. Rotorua, New Zealand. 1p.
- Bain, J. 1973. *Hylastes ater*. Forest Research Institute Unpublished Report. Rotorua, New Zealand. 1p.
- Bain, J. 1974. Overseas wood- and bark-boring insects intercepted at New Zealand ports. *Forest Research Institute Technical Paper No. 61*. Rotorua, New Zealand. 24p.
- Bain, J. 1977a. *Hylurgus ligniperda* (Fabricius) (Coleoptera: Scolytidae). *Forest and Timber Insects in New Zealand No. 18*. Forest Research Institute, New Zealand Forest Service. Rotorua, New Zealand. 8p.
- Bain, J. 1977b. *Pachycotes peregrinus* (Chapuis) (Coleoptera: Scolytidae). *Forest and Timber Insects in New Zealand No. 19*. Forest Research Institute, New Zealand Forest Service. Rotorua, New Zealand. 4p.
- Bain, J. 1980. *Hylastes ater* and establishment losses. Forest Research Institute Unpublished Report, New Zealand Forest Service. Rotorua, New Zealand. 1p.
- Bakshi, B. K. 1951a. Development of perithecia and reproductive structures in two species of *Ceratocystis*. *Annals of Botany* 57: 53-62
- Bakshi, B. K. 1951b. Studies on four species of *Ceratocystis*, with a discussion on fungi causing sap-stain in Britain. Commonwealth Mycological Institute. *Mycological Paper* 35: 1-16
- Ballard, R. G., Walsh, M. A. and Cole, W. E. 1982. Blue-stain fungi in xylem of lodgepole pine: a light-microscope study on the extent of hyphal distribution. *Canadian Journal of Botany* 60: 2334-2341
- Beaver, R. A. 1970. On *Hylastes ater* Payk. and *H. brunneus* Er. (Coleoptera: Scolytidae), with a description of the larvae of *H. ater*. *Entomologist*: 198-206
- Beaver, R. A. 1989. Insect-fungus relationships in the bark and ambrosia beetles. Pp 121-143 in Wilding, N., Collins, N.M., Hammond, P.M. and Webber, J.F. (eds) *Insect-fungus interactions. 14th symposium of the Royal Entomological Society of London in collaboration with the British Mycological Society*. Academic Press. London.
- Bedard, W. D., Ferrell, G. T., Whitmore, M. C. and Robertson, A. S. 1990. Trapping evaluation of beetle vectors of black stain root disease in Douglas-fir. *Canadian Entomologist* 122: 459-468
- Benade, E., Wingfield, M. J. and Van Wyk, P. S. 1995. Conidium development in the *Hyalorhinocladiella* anamorph of *Ophiostoma ips*. *Mycologia* 87: 298-303

- Berryman, A. A. 1972. Resistance of conifers to invasion by bark beetle-fungus associations. *Bioscience* 22: 598-602
- Berryman, A. A. 1974. Dynamics of bark beetle populations: towards a general productivity model. *Environmental Entomology* 3: 579-585
- Berryman, A. A. and Ashraf, M. 1970. Effects of *Abies grandis* resin on the attack behaviour and brood survival of *Scolytus ventralis* (Coleoptera: Scolytidae). *Canadian Entomologist* 102: 1229-1236
- Birch, M. 1978. Chemical communication in pine bark beetles. *American Scientist* 66: 409-419
- Birch, T. 1936. *Diplodia pinea* in New Zealand. *New Zealand State Forest Service Bulletin* No. 8. G.H. Loney, Government Printer, Wellington. 32p.
- Bois, E., Lieutier, F., Sauvard, D. and Yart, A. 1997. Phenolic and resistance of Scots pine to bark beetles. Pp 92-94 in Gregoire, J. C., Liebhold, A. M., Stephen, F. M., Day, K. R. and Salom, S. M. (eds) *Integrating cultural tactics into the management of bark beetle and reforestation pests*. General Technical Report NE-236. US Department of Agriculture, Forest Service, Northeastern Forest Experimental Station. Radnor, Pennsylvania USDA Forest Service.
- Boomsma, C. D. and Adams, A. J. S. 1943. The Pine bark beetle (*Hylastes ater*) at Mount Burr, South Australia. *Australian Forestry* 7: 33-37
- Borden, J. H. 1982. Aggregation pheromones. Pp 74-79 in Mitton, J. B. and Sturgeon, K. B. (eds) *Bark beetles in North American conifers*. University of Texas Press. Austin, USA.
- Borden, J. H. 1989. Semiochemicals and bark beetle populations: exploitation of natural phenomena by pest management strategists. *Holarctic Ecology* 12: 501-510
- Brasier, C. M. and Kirk, S. A. 1993. Sibling species within *Ophiostoma piceae*. *Mycological Research* 97: 811-816
- Brasier, C. M. and Stephens, T. M. 1993. Temperature-growth responses distinguish the OPC and OPH sibling species within '*Ophiostoma piceae*'. *Mycological Research* 97: 811-816
- Brasier, C. M. and Webber, J. F. 1991. Status of *Ophiostoma piceae* on hardwoods and conifers. Pp 47-48 in *Report on Forest Research 1990*, HMSO: London.
- Brattli, J. G., Andersen, J. and Nilssen, A. C. Primary attraction and host tree selection in deciduous and conifer living Coleoptera: Scolytidae, Curculionidae, Cerambycidae and Lymexylidae. *Journal of Applied Entomology* 122: 345-352

- Breuil, C., Seifert, K. A., Yamada, J., Rossignol, L. and Saddler, J. N. 1988. Quantitative estimation of fungal colonization of wood using an enzyme-linked immunosorbent assay. *Canadian Journal of Forest Research* 18: 374-377
- Bridges, J. R. 1995. Exotic pests: major threats to forest health. Pp 105-113 in Eskew, L. G. (compiler) *Forest health through silviculture: proceedings of the 1995 national silviculture workshop, Mescalero, New Mexico, May 8-11, 1995*. USDA Forest Service. General Technical Report, RM-GTR-267. Rocky Mountain Forest and Range Experiment Station. Fort Collins, Colorado.
- Brisson, A., Charibian, S., Eagen, R., Leclerc, D. and Breuil, C. 1996. Localization and characterization of the melanin granules produced by the sap-staining fungus *Ophiostoma piceae*. *Material und Organismen* 30: 23-32
- Burdon, R. D. 1992. Genetic survey of *Pinus radiata*. General discussion and implications for genetic management. *New Zealand Journal of Forestry Science* 22: 274-298
- Burdon, R. D. and Bannister, M. H. 1985. Growth and morphology of seedlings and juvenile cuttings in six populations of *Pinus radiata*. *New Zealand Journal of Forestry Science* 15: 123-34
- Burdon, R. D., Gaskin, R. E., Zabkiewicz, J. A. and Low, C. B. 1992a. Genetic survey of *Pinus radiata*. 7. Variation and inheritance of pinene composition in wood oleoresin. *New Zealand Journal of Forestry Science* 22: 246-256
- Burdon, R. D., Zabkiewicz, J. A. and Andrew, I. A. 1992b. Genetic survey of *Pinus radiata*. 8: Population differences in monoterpene composition of cortical oleoresin. *New Zealand Journal of Forestry Science* 22: 257-273
- Butcher, J. 1967. *Degrade by fungi of Pinus radiata posts during seasoning*. New Zealand Forest Service, Wellington.
- Butcher, J. 1968. The ecology of fungi infecting untreated sapwood of *Pinus radiata*. *Canadian Journal of Botany* 46: 1557-1589.
- Byers, J. A. 1989. Chemical ecology of bark beetles. *Experientia* 45: 271-283
- Byers, J. A. 1992. Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliatus*, and *Trypodendron domesticum* and other insects to short chain alcohols and monoterpenes. *Journal of Chemical Ecology* 18: 2385-2402
- Byers, J. A. 1995. Host tree chemistry affecting colonization in bark beetles. Pp 154-213 in Cardie, R.T. and Bell, W.J. (eds) *Chemical ecology of insects 2*. Chapman and Hall. New York.

- Cade, S. C., Hrutfiord, B. F. and Gara, R. I. 1970. Identification of a primary attractant for *Gnathotrichus sulcatus* isolated from western hemlock logs. *Journal of Economic Entomology* 63: 1014-1015
- Callahan, R. Z. 1964. Nature of resistance of pines to bark beetles. Pp 197-201 in Gerhold, H. D., Schreiner, E. J., McDermott, R. E. and Winieski, J. A. (eds) *Breeding pest resistant trees*. Pergamon Press, Oxford.
- Carson, M. J. 1986. Advantages of clonal forestry for *Pinus radiata*-real or imagined? *New Zealand Journal of Forestry Science* 16: 403-415
- Carson, M. J. and Inglis, C. S. 1988. Genotype and location effects on the internode length of *Pinus radiata* in New Zealand. *New Zealand Journal of Forestry Science* 18: 267-279
- Chapman, A. D. and Scheffer, T. C. 1940. Effect of blue stain on specific gravity and strength of southern pine. *Journal of Agricultural Research* 61: 125-133
- Chénier, J. V. R. and Philogène, B. J. R. 1989. Field responses of certain forest Coleoptera to conifer monoterpenes and ethanol. *Journal of Chemical Ecology* 15: 1729-1745
- Chou, C. K. S. 1976. A shoot die back in *Pinus radiata* caused by *Dothistroma pinea*. Inoculation studies. *New Zealand Journal of Forest Science* 6: 409-420
- Chou, C. K. S. 1984. *Diplodia* leader dieback, *Diplodia* crown wilt, *Diplodia* whorl canker. *Forest Pathology in New Zealand No 7*. Forest Research Institute. Rotorua, New Zealand. 4p.
- Christiansen, E., Waring, R. H. and Berryman, A. A. 1987. Resistance of conifers to bark beetle attack: searching for general relationships. *Forest Ecology and Management* 22: 89-106
- Ciesla, W. M. 1988. Pine bark beetles: a new pest management challenge for Chilean foresters. *Journal of Forestry*: 27-31
- Clark, A. F. 1930. *Forest biological research station: First annual report*. Department of Science and Industrial Research Unpublished Report. Rotorua, New Zealand. 1p.
- Clark, A. F. 1932a. Insects infesting *Pinus radiata* in New Zealand. *New Zealand Journal of Science and Technology* 13: 235-243
- Clark, A. F. 1932b. The pine-bark beetle, *Hylastes ater*, in New Zealand. *New Zealand Journal of Science and Technology* 14: 1-20
- Clark, A. F. 1937. *Hylastes ater* attacking apples. *New Zealand Journal of Forestry* 4: 98

- Cobb, F. W., Jr., Wood, D. L., Stark, R.W. and Parmeter Jr, J.R. 1968. Theory on the relationships between oxidant injury and bark beetle infestation. *Hilgardia* 39: 127-134
- Coulson, R. N. 1979. Population dynamics of bark beetles. *Annual Review of Entomology* 24: 417-447
- Crowhurst, P. S. 1969. Observations on the breeding behaviour of *Hylastes ater* Paykull (Scolytidae: Coleoptera) at Eyrewell Forest. *Forest Entomology Report No 23*. (unpublished) New Zealand Forest Research Institute. Rotorua New Zealand. 23p.
- Crowson, R. A. 1968. *A natural classification of the families of Coleoptera*. E.W. Classey Ltd. Hampton, England. 195p.
- Dale, P. S. 1967. Nematodes associated with the pine-bark beetle, *Hylastes ater*, in New Zealand. *New Zealand Journal of Science* 10: 222-234
- De Jong, M. C. M. and Sabelis, M. W. 1988. How bark beetles avoid interference with squatters: an ESS for colonisation by *Ips typographus*. *Oikos* 51: 88-96
- Delorme, J. D. and Payne, T. L. 1990. Antennal olfactory responses of black turpentine beetle, *Dendroctonus terebrans* (Oliver), to bark beetle pheromones and host terpenes. *Journal of Chemical Ecology* 16: 1321-1329
- Dick, M. 1998. Pine pitch canker-the threat to New Zealand. *New Zealand Forestry* 42: 30-34
- Dick, M and Bain, J. 1996. *Pine pitch canker: The risk to New Zealand*. Forest Research Institute unpublished report, Rotorua. 35p.
- Dowding, P. 1973. Effects of felling time and insecticide treatment on the interrelationships of fungi and arthropods in pine logs. *Oikos* 24: 422-429
- Dowding, P. 1984. The evolution of insect-fungus relationships in the primary invasion of forest timber. Pp 133-153 in Andersen, J. M., Rayner, A. D. M. and Walton, D. W. H. (eds) *Invertebrate-microbial interactions*. Cambridge University Press.
- Du Toit, P. F. 1975. Aspekte van die bio-ekologie en bestryding van die baskewer, *Hylastes angustatus* Herbst (Coleoptera: Scolytidae). *Forestry in South Africa* 16: 37-43
- Duffy, E. A. J. 1953. Coleoptera, Scolytidae and Platypodidae. *Handbook of the Identification of British Insects* 5 (pt. 15): 1-20
- Dugdale, J. S. and Zondag, R. 1966. *Symposium on the role of other pines in New Zealand forestry, March 1966. Pt. section 7: Insects*. Forest Service Forest Research Institute. Rotorua, New Zealand. 8p.

- Eagen, R., Brisson, A. and Breuil, C. 1997. The sap-staining fungus *Ophiostoma piceae* synthesizes different types of melanin in different growth media. *Canadian Journal of Microbiology* 43: 592-595
- Eidmann, H. H. 1992. Impact of bark beetles on forests and forestry in Sweden. *Journal of Applied Entomology* 114: 193-200
- Eidmann, H. H. and von Sydow, F. 1989. Stockings for protection of containerised conifer seedlings against pine weevil (*Hylobius abietis* L.) damage. *Scandinavian Journal of Forest Research* 4: 537-547
- Eidmann, H. H., Kula, E. and Lindelöw, Å. 1991. Host recognition and aggregation behaviour of *Hylastes cunicularius* Erichson (Col., Scolytidae) in the laboratory. *Journal of Applied Entomology* 112: 11-18
- Eidmann, H. H., Nordenhem, H. and Weslien, J. 1996. Physical protection of conifer seedlings against pine weevil feeding. *Scandinavian Journal of Forest Research* 11: 68-75
- Fabre, J. P. and Carle, P. 1975. Contribution a L'étude Biologique D'*Hylurgus ligniperda* F. (Coleoptera: Scolytidae) dans le Sud-Est se la France. *Annales Scientifique Forestieres* 32: 55-71
- Farrell, R. L., Hadar, E., Kay, S. J., Blanchette, R. A. and Harrington, T. C. 1997. Survey of sapstain organisms in New Zealand and albino anti-sapstain fungi. Pp 57-62 in *Biology and Prevention of sapstain*. Proceedings of conference, Whistler, Canada, May 25, 1997.
- Faulds, W. 1989. *Hylastes ater* (Paykull), black pine bark beetle and *Hylurgus ligniperda* (Fabricius), golden haired bark beetle. Pp 271-275 in Cameron, P. J., Hill, R. L., Bain, J. and Thomas, W. P. (eds) *A review of biological control of pests and weeds in New Zealand 1874 to 1987*. Technical Communication No. 10. C.A.B. International & DSIR. Wallingford, UK.
- Flectmann, C. A. H., Dalusky, M. J. and Beristord, C. W. 1999. Bark and ambrosia beetles (Coleoptera: Scolytidae) responses to volatiles from ageing loblolly pine billets. *Environmental Entomology* 28: 638-648
- Forestry Commission. 1946. *The black Pine beetle (Hylastes ater) and other closely allied beetles*. Leaflet No 4. London. 9p.
- Fox, J. W., Wood, D. L., Koehler, C. S. and O'Keefe, S. T. 1991. Engraver beetles (Scolytidae: *Ips* species) as vectors of the pitch canker fungus, *Fusarium subglutinans*. *Canadian Entomologist* 123: 1355-1367

- Franklin, R. T. and Taylor, J. W. Jr. 1970. Biology of *Pachylobius picivorus* (Coleoptera: Curculionidae) in the Georgia Piedmont. *Canadian Entomologist* 102: 962-968
- Gao, Y. and Breuil, C. 1995. Extracellular lipase production by a sapwood-staining fungus, *Ophiostoma piceae*. *World Journal of Microbiology and Biotechnology* 11: 638-642
- Gara, R. I. and Vite, J. P. 1962. Studies on the flight patterns of bark beetles (Coleoptera: Scolytidae) in second growth Ponderosa Pine forests. *Contributions from the Boyce Thompson Institute* 21: 275-289
- Gibbs, J. N. 1993. The biology of Ophiostomatoid fungi causing sapstain in trees and freshly cut logs. Pp153-160 in Winfield, M. J., Seifert, K. A. and Webber, J. F. (eds) *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. American Phytopathological Society, St. Paul, Minnesota.
- Goheen, D. J. and Cobb Jr, F. W. 1978. Occurrence of *Verticicladiella wagnerii* and its perfect state, *Ceratocystis wagnerii* sp. nov. in insect galleries. *Phytopathology* 68: 1192-1195
- Goheen, D. J. and Hansen, E. M. 1993. Effects of pathogens and bark beetles on forests. Pp 175-193 in Schowalter, T.D. and Filip, G.M. (eds) *Beetle-pathogen interactions in conifer forests*. Academic Press, London.
- Graham, K. 1959. Release by flight exercise of the chemotropic response from photopositive domination in a scolytid beetle. *Nature* 184: 282-284
- Grégoire, J. C., Raty, L., Drumont, A. and De Windt, N. 1997. Pheromone mass trapping: does it protect windfalls from attack by *Ips typographus* L. (Coleoptera: Scolytidae)? Pp 1-8 in Gregoire, J. C., Liebhold, A. M., Stephen, F. M., Day, K. R. and Salom, S. M. (eds) *Integrating cultural tactics into the management of bark beetle and reforestation pests*. General Technical Report NE-236. US Department of Agriculture, Forest Service, Northeastern Forest Experimental Station. Radnor, Pennsylvania USDA Forest Service.
- Griffen, H. 1968. The genus *Ceratocystis* in Ontario. *Canadian Journal of Botany* 46:689-717
- Grocholski, J., Michalski, W. and Novak, W. 1977. Some notes on the morphology and occurrence of the brown pine beetle-*Hylastes brunneus* Er. (Coleoptera, Scolytidae). *Bulletin Entomologique de Pologne* 47: 703-710
- Hagner, M. and Jonsson, C. 1995. Survival after planting without soil preparation for pine and spruce seedlings protected from *Hylobius abietis* by physical and chemical shelters. *Scandinavian Journal of Forest Research* 19: 225-234

- Halmschlager, E., Messner, R. Kowalski, T. and Prillinger, H. 1994. Differentiation on *Ophiostoma piceae* and *Ophiostoma quercus* by morphology and RAPD analysis. *Systematic and Applied Microbiology* 17: 554-562
- Hanover, J. W. 1975. Physiology of tree resistance to insects. *Annual Review of Entomology* 20: 75-95
- Hansen, V. 1955. Notes on some species of *Hylastes* Er. and *Trypophloeus* Fairm (Coleopt. Scolytidae). *Entomologiske Meddelelser* 27: 169-185
- Harrington, T. C. 1988. *Leptographium* species, their distributions, hosts and insect vectors. Pp 1-39 in Harrington, T. C. and Cobb, F. W. Jr. (eds) *Leptographium root diseases of conifers*. American Phytopathological Society Press. St Paul, Minnesota.
- Harrington, T. C. 1993a. Biology and taxonomy of fungi associated with bark beetles. Pp-37-58 in Schowalter, T. D. and Filip, G. M. (eds) *Beetle-pathogen interactions in conifer forests*. Academic Press, London.
- Harrington, T. C. 1993b. Diseases of conifers caused by species of *Ophiostoma* and *Leptographium*. Pp 161-172 in Winfield, M. J., Seifert, K. A. and Webber, J. F. (eds) *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. American Phytopathological Society, St. Paul, Minnesota.
- Harrington, T. C. and Cobb, F. W. Jr. 1983. Pathogenicity of *Leptographium* and *Verticicladiella* spp. isolated from roots of western North American conifers. *Phytopathology* 74: 286-290
- Harrington, T. C. and Wingfield, M. J. 1998. Diseases and the ecology of indigenous and exotic pines. Pp 381-404 in Richardson, D. M. (ed) *Ecology and biogeography of Pinus*. Cambridge University Press, Cambridge.
- Harrington, T. C., Cobb, F. W. Jr. and Lownsbery, J. W. 1985. Activity of *Hylastes nigrinus*, a vector of *Verticicladiella wagnerii*, in thinned stands of Douglas-fir. *Canadian Journal of Forest Research* 15: 519-523
- Harrington, T. C., McNew, D., Steimel, J., Hofstra, D. and Farrell, R.L. 2001. Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch Elm disease fungi. *Mycologia*: in press
- Hausner, G., Reid, J. and Klassen, G. R. 2000. On the phylogeny of members of *Ceratocystis* s.s and *Ophiostoma* that possess different anamorphic states, with emphasis on the anamorph genus *Leptographium*, based on partial ribosomal DNA sequences. *Canadian Journal of Botany* 78: 903-916

- Heikkinen, H. J. 1977. Southern pine beetle: a hypothesis regarding its primary attractant. *Journal of Forestry*: 412-413
- Heikkinen, H. J. and Hrutford, B. J. 1965. *Dendroctonus pseudotsugae*: a hypothesis regarding its primary attractant. *Science* 150: 1457-1459
- Hepting, G. H. and Roth, E. R. 1946. Pitch Canker, a new disease of some southern pines. *Journal of Forestry* 44: 742-744
- Heritage, S. 1997. *Protecting plants from weevil damage by dipping or spraying before planting using aqueous insecticides*. Research Information Note 270. Farnham, Forestry Commission Research Division. 4p.
- Heritage, S. and Johnson, D. 1997. *The use of post-planting sprays to improve the protection of plants from damage by Hylobius abietis*. Research Information Note 272. Farnham, Forestry Commission Research Division. 5p.
- Heritage, S. G., Collins, S. and Evans, H. F. 1989. A survey of damage by *Hylobius abietis* and *Hylastes* spp. in Britain. Pp 28-33 in Alfaro, R. L. and Glover, S. G. (eds) *Insects affecting reforestation: biology and damage*. Victoria, Canada, Pacific and Yukon Region, Forestry Canada.
- Heritage, S., Johnson, D. and Jennings, T. 1997a. *The use of Marshal/suSCon granules to protect plants from Hylobius damage*. Research Information Note 269. Farnham, Forestry Commission Research Division. 4p.
- Heritage, S., Johnson, D. and Jennings, T. 1997b. *The use permethrin 12ED through the electrodyn sprayer conveyor to protect plants from Hylobius damage*. Research Information Note 271. Farnham, Forestry Commission Research Division. 4p.
- Hermes, D. A. and Mattson, W. J. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* 67: 283-335
- Hertert, H. D., Miller, D. L. and Partridge, A. D. 1975. Interaction of bark beetles (Coleoptera: Scolytidae) and root-rot pathogens in grand fir in northern Idaho. *Canadian Entomologist* 107: 899-904
- Hines, J. W. and Heikkinen, H. J. 1977. Beetle attracted to severed Virginia pine (*Pinus virginiana* Mill.). *Environmental Entomology* 6: 123-127
- Hobson, K.R. 1992. *Studies on host selection and biology of Dendroctonus valens and fungal host interactions of Dendroctonus brevicomis*. Ph.D. dissertation, University of California, Berkeley. 65p.

Hobson, K. R., Wood, D. L., Cool, L. G., White, P. R., Toshikazu, O., Isao, K. and Eugene, Z. 1993. Chiral specificity in responses by the bark beetle *Dendroctonus valens* to host kairomones. *Journal of Chemical Ecology* 19: 1837-1846

Zavodny, E. Hodges, J. D., Elam, W. W., Watson, W.F. and Nebeker, T. E. 1979. Oleoresin characteristics and susceptibility of four southern pines to southern pine beetle (Coleoptera: Scolytidae) attacks. *Canadian Entomologist* 111: 889-896

Hosking, G. P. 1977. Insect survey in Canterbury windthrow. *What's New in Forest Research No. 48*. Forest Research Institute. Rotorua, New Zealand. 4p.

Hosking, G. P. 1979. *Xyleborus saxeseni* (Ratzenburg) (Coleoptera: Scolytidae) A keyhole ambrosia beetle. *Forest and Timber Insects in New Zealand No. 39*. New Zealand Forest Service, Forest Research Institute. Rotorua, New Zealand. 4p.

Hunt, D. W. A., Borden, J. H., Lindgren, B. S. and Gries, G. 1989. The role of autoxidation of α -pinene in the production of pheromones of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *Canadian Journal of Forest Research* 19: 1275-1282

Hunt, J. 1956. Taxonomy of the genus *Ceratocystis*. *Lloydia* 19: 1-58

Jacobi, W. R. 1992. Potential insect vectors of the black stain root disease pathogen on Southern Vancouver Island. *Journal of the Entomological Society of British Colombia* 89: 54-56

Jacobs, K., Wingfield, M. J., Wingfield, B. D. and Yamaoka, Y. 1998. Comparison of *Ophiostoma huntii* and *O. europioides* and description of *O. aenigmaticum* sp. nov. *Mycological Research* 102: 289-294

Jayawickrama, K. J. S., Shelbourne, C. J. A. and Carson, M. J. 1997. New Zealand's long internode breed of *Pinus radiata*. *New Zealand Journal of Forestry Science* 27: 126-141

Kalkstein, L. S. 1981. An improved technique to evaluate climate-southern pine beetle relationships. *Forest Science* 27: 579-589

Kay, S. J., Farrell, R. L., Hader, E., Hader, Y., Blanchette, R. A. and Harrington, T. C. 1997. Sapstain in New Zealand-the causes and a potential anti-sapstain solution. *Proceedings of the 11th Biennial Conference of the Australasian Plant Pathology Society*. 21p.

Kirkendall, L. R. 1983. The evolution of mating systems in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). *Zoological Journal of the Linnean Society* 77: 293-352

- Kirkendall, L. R., Kent, D. S. and Raffa, K. F. 1997. Interactions among males, females and offspring in bark and ambrosia beetles: the significance of living in tunnels for the evolution of social behaviour. Pp 181-215 in Choe, J. C. and Cuespi, B. J. (eds) *The evolution of social behaviour in insects and arachnids*. Cambridge University Press, Cambridge.
- Klepzig, K. D. 1998. Competition between a biological control fungus, *Ophiostoma piliferum*, and symbionts of the southern pine beetle. *Mycologia* 90: 69-75
- Klepzig, K. D. and Schlyter, F. 1999. Laboratory evaluation of plant derived antifeedants against pine weevil *Hylobius abietis* twig feeding. *Journal of Economic Entomology* 92: 644-650
- Klepzig, K. D., Raffa, K. F. and Smalley, E. B. 1991. Association of an insect-fungal complex with red pine decline in Wisconsin. *Forest Science* 37: 1119-1139
- Klepzig, K. D., Smalley, E. B. and Raffa, K. F. 1996a. Combined chemical defences against an insect-fungal complex. *Journal of Chemical Ecology* 22: 1367-1388
- Klepzig, K. D., Smalley, E. B. and Raffa, K. F. 1996b. Interactions of ecologically similar saphrogenic fungi with healthy and abiotically stressed conifers. *Forest Ecology and Management* 86: 163-169
- Kuschel, G. 1990. *Beetles in a suburban environment: a New Zealand case study. The identity and status of Coleoptera in the natural and modified habitats of Lynfield, Auckland (1974-1989)*. Department of Science and Industrial Research. Plant Protection Report no. 3. 118p.
- Kuschel, G. 1995. A phylogenetic classification of Curculionidae to families and subfamilies. *Memoirs of the Entomological Society of Washington* 14: 5-35
- Larsson, S., Oren, R., Waring, R. H. and Barrett, J. W. 1983. Attacks of mountain pine beetle as related to tree vigor of ponderosa pine. *Forest Science* 29: 395-402
- Lawson, S. A. and Morgan, F. D. 1993. Prey specificity of adult *Temnochila virescens* F. (Col., Trogositidae), a predator of *Ips grandicollis* Eichh. (Col., Scolytidae). *Journal of Applied Entomology* 115: 139-144
- Leach, J. B. 1940. *Insect transmission of plant diseases*. McGraw-Hill Book Co. New York. 615p.
- Leather, S. R., Day, K. R. and Salisbury, A. N. 1999. The biology and ecology of the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae): a problem of dispersal? *Bulletin of Entomological Research* 89: 3-16

- Leather, S. R., Small, A. A. and Bøgh, S. 1995. Seasonal variation in local abundance of adults of the large pine weevil, *Hylobius abietis* L. (Col., Curculionidae). *Journal of Applied Entomology* 119: 511-513
- Lekander, B. 1965. On *Hylastes ater* Payk. and *Hylastes brunneus* Er. (Col. Scolytidae). *Entomologisk Tidskrift* 86: 184-195
- Lekander, B., Bejer-Petersen, B. Kangas, E. and Bakke, A. 1977. The distribution of bark beetles in the Nordic countries. *Acta Entomologica Fennica* 32: 1-36
- Lemperière, G. and Julien, J. M. 1989. Premiers résultats de tests pour l'évaluation de l'efficacité d'un insecticide systémique contre l'hylobe (*Hylobius abietis* L., Col. Curculionidae). *Technique et Forêt* 41: 411-422
- Lévieux, J., Piou, D., Cassier, P., André, M. and Guillaumin, D. 1994. Association of phytopathogenic fungi for the scots pine (*Pinus sylvestris* L.) with the European pine weevil *Hylobius abietis* (L.) (Col. Curculionidae). *Canadian Entomologist* 126: 929-936
- Lewis, K. J. and Alexander, S. A. 1986. Insects associated with the transmission of *Verticicladiella procera*. *Canadian Journal of Forest Research* 16: 1330-1333
- Lieutier, F. 1993. Induced defence reaction of conifers to bark beetles and their associated *Ophiostoma* species. Pp 225-233 in Winfield, M. J., Seifert, K. A. and Webber, J. F. (eds) *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. American Phytopathological Society, St. Paul, Minnesota.
- Lieutier, F., Brignolas, F., Sauvard, D., Galet, C., Yart, A., Brunet, M., Christiansen, E., Solheim H. and Berryman A. 1997a. Phenolic compounds as predictors of Norway spruce resistance to bark beetles. Pp 215-216 in Gregoire, J. C., Liebhold, A. M., Stephen, F. M., Day, K. R. and Salom, S. M. (eds) *Integrating cultural tactics into the management of bark beetle and reforestation pests*. General Technical Report NE-236. US Department of Agriculture, Forest Service, Northeastern Forest Experimental Station. Radnor, Pennsylvania USDA Forest Service.
- Lieutier, F., Garcia, J., Yart, A., Gerard, L., Malphettes, C. B. and Romary, P. 1996. Preliminary investigations on the relationship between phloem phenolic content of scots pine and maturation feeding of the pine weevil *Hylobius abietis*. Pp 73-79 in Gregoire, J. C., Liebhold, A. M., Stephen, F. M., Day, K. R. and Salom, S. M. (eds) *Integrating cultural tactics into the management of bark beetle and reforestation pests*. General Technical Report NE-236. US Department of Agriculture, Forest Service,

- Northeastern Forest Experimental Station. Radnor, Pennsylvania USDA Forest Service.
- Lindelöw, Å. 1992. *Hylastes cunicularius*: host orientation, impact of feeding in spruce plantations, and population sizes in relation to seedling mortality (Coleoptera: Scolytidae). Ph.D. dissertation, Sveriges Lantbruksuniversitet, Uppsala. 41p.
- Lindelöw, Å., Eidmann, H. H. and Nordenhem, H. 1993. Response on the ground of bark beetle and weevil species colonising conifer stumps and roots to terpenes and ethanol. *Journal of Chemical Ecology* 19: 1393-1403
- Lindelöw, Å., Risberg, B. and Sjödin, K. 1992. Attraction during flight of scolytids and other bark- and wood-dwelling beetles to volatiles from fresh and stored spruce wood. *Canadian Journal of Forestry Research* 22: 224-228
- Lindgren, B. S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). *Canadian Entomologist* 115: 299-302
- Lindgren, B. S., Norlander, G. and Birgersson, G. 1996. Feeding deterrence of verbenone to the pine weevil, *Hylobius abietis* (L.) (Col., Curculionidae). *Journal of Applied Entomology* 120: 397-403
- Lindstrom, A., Hellqvist, C., Gyldberb, B., Långström, B. and Mattsson, A. 1986. Field performance of a protective collar against damage by *Hylobius abietis*. *Scandinavian Journal of Forest Research* 1: 3-15
- Lorio, P. L. 1968. Soil and stand conditions related to southern pine beetle activity in Hardin County, Texas. *Journal of Economic Entomology* 61: 565-567
- Lorio, P. L. 1994. The relationship of oleoresin exudation pressure (or lack thereof) to flow from wounds. *Journal of Sustainable Forestry* 1: 81-93
- Lorio, P. L. and Sommers, R. A. 1986. Evidence of competition for photosynthates between growth processes and oleoresin synthesis in *Pinus taeda* L. *Tree Physiology* 2: 301-306
- Lownsbery, J. W. 1988. *Factors affecting the abundance and activity of Hylastes nigrinus, Steremnius carinatus and other insects in roots of Douglas-fir stumps*. Ph.D. dissertation. Graduate Division. University of California, Berkeley. 130p.
- Löyttyniemi, K. & Hiltunen, R. 1976. The effect of nitrogen fertilization and terpene content on the attractiveness of pine trapping bolts to *Hylobius abietis* L. and *Pissodes pini* F. (Col., Curculionidae). *Communicationes Instituti Forestalis Fenniae* 88.6. 19p.

- Löyttyniemi, K., Heliövaara, K. and Repo, S. 1988a. No evidence of a population pheromone in *Tomicus piniperda* (Coleoptera, Scolytidae): a field experiment. *Annales Entomologici Fennici* 54: 93-95
- Löyttyniemi, K., Heliövaara, K. and Repo, S. 1988b. Pine turpentine strongly attracts *Hylastes brunneus* (Coleoptera, Scolytidae). *Annales Entomologici Fennici* 54: 145-148
- MacKenzie, M. & Dick, M. 1984. *Verticicladiella* root disease. *Forest Pathology in New Zealand* No. 6. Forest Research Institute. New Zealand Forest Service. Rotorua, New Zealand. 4p.
- MAF 2000. *Catalogue marker pest list for plants and plant products*. Version 2 May 2000. Ministry of Agriculture and Forestry, Biosecurity Authority (Plants). Wellington.
- Malloch, D. and Blackwell, M. 1993. Dispersal biology of the Ophiostomatoid fungi. Pp 195-205 in Winfield, M. J., Seifert, K. A., and Webber, J. F. (eds) *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. American Phytopathological Society, St. Paul, Minnesota.
- Manlove, J. D., Styles, J. and Leather, S. R. 1997. Feeding of the adults of the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae). *European Journal of Entomology* 94: 153-156
- Mason, R. R. 1971. Soil moisture and stand density affect oleoresin exudation in a loblolly pine plantation. *Forest Science* 17: 170-177
- Mathiesen-Käärik, A. 1960. Studies on the ecology, taxonomy and physiology of Swedish insect-associated blue stain fungi, especially the genus *Ceratocystis*. *Oikos* 11: 1-25
- Matson, P. A., Hain, F. P. and Mawby, W. 1987. Indices of tree susceptibility to bark beetles vary with silvicultural treatment in a loblolly pine plantation. *Forest Ecology and Management* 22: 107-118
- May, B. M. 1993. Larvae of the Curculionidae (Insecta: Coleoptera): a systematic overview. *Fauna of New Zealand* No. 28. Manaaki Whenua Press. New Zealand. 226p.
- Maynard Smith, J. 1989. *Evolutionary genetics*. Oxford University Press. Oxford. 325p.
- McCain, A. H., Koehler, C. S. and Tjosvold, S. A. 1987. Pitch canker threatens Californian pines. *California Agriculture* 41: 22-23
- Mendel, Z., Boneh, O. and Riov, J. 1992. Some foundations for the application of aggregation pheromone to control pine bark beetles in Israel. *Journal of Applied Entomology* 114: 217-227

- Miller, D. 1935. Control of forest insect pests: distribution of parasites in New Zealand. *New Zealand Journal of Science and Technology* 16: 301-307
- Milligan, R. H. 1971a. *Hylastes ater*. New Zealand Forest Research Institute Unpublished Report. Rotorua, New Zealand. 9p.
- Milligan, R. H. 1971b. *Hylastes establishment failures at Karioi Forest*. New Zealand Forest Research Institute Unpublished Report. Rotorua, New Zealand. 4p.
- Milligan, R. H. 1978. *Hylastes ater* (Paykull) (Coleoptera: Scolytidae): black pine beetle. *Forest and Timber Insects in New Zealand No. 29*. Forest Research Institute, New Zealand Forest Service. Rotorua, New Zealand. 8p.
- Mitchell, R. G., Waring, R. H. and Pitman, G. B. 1983. Thinning lodgepole pine increases tree vigor and resistance to mountain pine beetle. *Forest Science* 29: 204-211
- Moeck, H. A. 1970. Ethanol as the primary attractant for the ambrosia beetle *Trypodendron lineatum* (Coleoptera: Scolytidae). *Canadian Entomologist* 102: 985-995
- Mrlina, G., Lemperière, G. and Calmon, J. P. 1994. Determination and uptake of carbosulfan and carbofuran in young Douglas-firs (*Pseudotsuga menziesii* Mirb.). *Journal of Agricultural and Food Chemistry* 42: 1569-1571
- Munro, J. W. 1917. The genus *Hylastes*, Er. *Proceedings of the Royal Physical Society of Edinburgh* 20: 123-158
- Munro, J. W. 1926. British bark beetles. *Forestry Commission Bulletin No 8*. London. 77p.
- Munro, J. W. 1929. The biology and control of *Hylobius abietis* L. part II. *Forestry* 3: 61-65
- Nebeker, T. E., Hodges, J. D. and Blanche, C. A. 1993. Host response to bark beetle and pathogen colonisation. Pp 157-171 in Schowalter, T. D. and Filip, G. M. (eds) *Beetle-pathogen interactions in conifer forests*. Academic Press, London.
- Neumann, F. G. 1987. Introduced bark beetles on exotic trees in Australia with special reference to *Ips grandicollis* in pine plantations. *Australian Forestry* 50: 166-178
- Nevill, R. J. and Alexander, S. A. 1992. Transmission of *Leptographium procerum* to eastern white pine by *Hylobius pales* and *Pissodes nemorensis* (Coleoptera: Curculionidae). *Plant Disease* 76: 307-310
- New Zealand Forestry Corporation Limited. 1989. *Sale of State owned forests in New Zealand*. Resource description southern North Island (unpublished report).
- New Zealand Soil Bureau. 1968. Soils of New Zealand. *New Zealand Soil Bureau Bulletin* 26. Part 1 and 2. 362p.

- Niemeyer, H. 1997. Integrated bark beetle control: experiences and problems in Northern Germany. Pp 80-86 in Gregoire, J. C., Liebhold, A. M., Stephen, F. M., Day, K. R. and Salom, S. M. (eds) *Integrating cultural tactics into the management of bark beetle and reforestation pests*. General Technical Report NE-236. US Department of Agriculture, Forest Service, Northeastern Forest Experimental Station. Radnor, Pennsylvania USDA Forest Service.
- Nilssen, A. C. 1984. Long-range aerial dispersal of bark beetles and bark weevils (Coleoptera, Scolytidae and Curculionidae) in northern Finland. *Annales Entomologici Fennici* 50: 37-42
- Nord, J. C., Ragenovich, I. and Doggett, C. A. 1984. Pales Weevil. U.S. Department of Agriculture Forest Service. *Forest Insect and Disease Leaflet* 104. 7p.
- Nordenhem, H. 1989. Age, sexual development, and seasonal occurrence of the pine weevil *Hylobius abietis* (L.). *Journal of Applied Entomology* 108: 260-270
- Norlander, G. 1987. A method for trapping *Hylobius abietis* (L.) with a standardized bait and its potential for forecasting seedling damage. *Scandinavian Journal of Forest Research* 2: 199-213
- Norlander, G. 1989. The use of artificial baits to forecast seedling damage caused by *Hylobius abietis* (Coleoptera: Curculionidae). Pp 34-35 in Alfaro, R. I. and Glover, S. G. (eds) *Insects affecting reforestation: biology and damage*. Victoria, Canada, Pacific and Yukon Region, Forestry Canada.
- Norlander, G., Eidmann, H. H., Jacobsson, U., Nordenhem, H. and Sjödin, K. 1986. Orientation of the pine weevil *Hylobius abietis* to underground sources of host volatiles. *Entomologia Experimentalis et Applicata* 41: 91-100
- Örlander, G. and Nilsson, U. 1999. Effect of restoration methods on pine weevil (*Hylobius abietis*) damage and seedling survival. *Scandinavian Journal of Forest Research* 14: 341-354
- Örlander, G., Nilsson, U. and Nordlander, G. 1997. Pine weevil abundance on clear-cuttings of different ages: a 6-year study using pitfall traps. *Scandinavian Journal of Forest Research* 12: 225-240
- Paine, T. D., Birch, M. C. and Švihra, P. 1981. Niche breadth and resource partitioning by four sympatric species of bark beetles (Coleoptera: Scolytidae). *Oecologia* 48: 1-6
- Paine, T. D., Raffa, K. F. and Harrington, T. C. 1997. Interactions among Scolytid bark beetles, their associated fungi, and live host conifers. *Annual Review of Entomology* 42: 179-206

- Paine, T. D., Stephen, F. M. and Cates, R. G. 1993. Host defence reactions in response to inoculation with *Ophiostoma* species. Pp 219-224 in Winfield, M. J., Seifert, K. A. and Webber, J. F. (eds) *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. American Phytopathological Society, St. Paul, Minnesota.
- Pearson, H. L. 1931. Theory in explanation of the selection of certain trees by the western pine beetle. *Journal of Forestry* 29: 696-699
- Perttunen, V. 1957. Reactions of two bark beetle species, *Hylurgops palliatus* Gyll. and *Hylastes ater* Payk. (Col., Scolytidae) to the terpene alpha-pinene. *Annual Review of Entomology* 23: 101-110
- Perttunen, V. K., Kangas, E. Oksanen, H. 1968. The mechanisms by which *Blastophagus* (*Myelophilus*) *piniperda* (Col. Scolytidae) reacts to the odour of an attractant fraction isolated from Pine phloem. *Annales Entomologici Fennici* 34: 205-222
- Phillips, T. W. 1990. Responses of *Hylastes salebrosus* to turpentine, ethanol, and pheromones of *Dendroctonus* (Coleoptera: Scolytidae). *Florida Entomologist* 73: 286-292
- Phillips, T. W., Wilkening, A. J., Atkinson, T. H., Nation, J. L., Wilkinson, R. C. and Foltz, J. L. 1988. Synergism of turpentine and ethanol as attractants for certain pine-infesting beetles (Coleoptera). *Environmental Entomology* 17: 456-462
- Pipe, N. D., Buck, K. W. and Brasier, C. M. 1995. Genomic fingerprinting supports the separation of *Ophiostoma piceae* into two species. *Mycological Research* 99: 1182-1186
- Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* 64: 244-251
- Quayle, A. M. 1984. The climate and weather of the Bay of Plenty region. *New Zealand Meteorological Service Miscellaneous Publication* 115(1). 2nd edition. 56p.
- Raffa, K. F. 1991. Induced defensive reactions in conifer-bark beetle systems. Pp 246-276 in Tallamy, D. W. and Raupp, M. J. (eds) *Phytochemical induction by herbivores*. John Wiley and Sons Inc., USA.
- Raffa, K. F. and Berryman, A. A. 1983. The role of host plant resistance in the colonisation behaviour and ecology of bark beetles (Coleoptera; Scolytidae). *Ecological Monographs* 53: 27-49
- Raffa, K. F., Phillips, T. W. and Salom, S. M. 1993. Mechanisms and strategies of host colonisation by bark beetles. Pp 103-128 in Schowalter, T. O. and Filip, G. M. (eds) *Interactions among bark beetles, pathogens, and conifers in North American forests*. Academic Press Ltd., London.

- Ranta, H., Pulkkinen, P. and Neuvonen, S. 2000. Susceptibility of six scots pine clones to the pathogenic fungus *Gremmeniella abietina*. *Scandinavian Journal of Forest Research* 15: 7-12
- Rawlings, G. B. 1959. Problems of forest entomology in exotic forests in New Zealand. *Proceedings of the tenth International Congress of Entomology, Montreal, 1956* 22: 241-247
- Rawlings, G. B. 1961. Entomology and other factors in the ecology of a *Pinus radiata* plantation. *Proceedings of the New Zealand Ecological Society* 8: 47-51
- Reitter, E. 1913. Bestimmungs-Tabelle der Borkenkäfer (Scolytidae) aus Europa und den angrenzenden Ländern. *Wiener Entomologische Zeitung* 32: 1-116
- Rieske, L. K. 2000. Pine weevil (Coleoptera: Curculionidae) population monitoring in Christmas trees using volatile host compounds. *Journal of Entomological Science* 35: 167-175
- Rieske, L. K. and Raffa, K. F. 1990. Dispersal patterns and mark-and-recapture estimates of two pine root weevil species, *Hylobius pales* and *Pachylobius picivorus* (Coleoptera: Curculionidae), in Christmas tree plantations. *Environmental Entomology* 19: 1829-1836
- Rieske, L. K. and Raffa, K. F. 1991. Effects of varying ethanol and turpentine levels on attraction of two pine root weevil species, *Hylobius pales* and *Pachylobius picivorus* (Coleoptera: Curculionidae). *Environmental Entomology* 20: 48-52
- Rieske, L. K. and Raffa, K. F. 1999. Baited pitfall traps and evaluation of dispensing methods for root weevils (Coleoptera: Curculionidae) in newly established pine plantations in Wisconsin. *Journal of Economic Entomology* 92: 439-444
- Rijske, W. 1994. *Soils of the Kinleith Forests central North Island New Zealand*. Landcare Research Contract report (unpublished). 121p.
- Robinson-Jeffery, R. C. and Grinchenko, A. H. H. 1964. A new fungus in the genus *Ceratocystis* occurring on blue-stained lodgepole pine by bark beetles. *Canadian Journal of Botany* 46: 1523-1527
- Rudinsky, J. A. 1962. Ecology of Scolytidae. *Annual review of Entomology* 7: 327-348
- Rudinsky, J. A. and Vité J. P. 1956. Effects of temperature upon the activity and the behaviour of the Douglas-fir beetle. *Forest Science* 2: 258-267
- Rudinsky, J. A. and Zethner-Møller, O. 1967. Olfactory responses of *Hylastes nigrinus* (Coleoptera: Scolytidae) to various host materials. *Canadian Entomologist* 99: 911-916

- Rumbold, C. T. 1931. Two blue-staining fungi associated with bark-beetle infestation of pines. *Journal of Agricultural research* 43: 847-873
- Sahota, T. S., Manville, J. F. and White, E. 1994. Interaction between Sitka spruce weevil and its host, *Picea sitchensis* (Bong) Carr.: a new mechanism for resistance. *Canadian Entomologist* 126: 1067-1074
- Salom, S. M. 1997. Pest status and management of *Hylobius pales* in North America. *Tree Planters Notes* 48: 4-11
- Salom, S. M., Carlson, J. A., Ang, B. N., Grosman, D. M. and Day, E. R. 1994. Laboratory evaluation of biologically-based compounds as antifeedants for the pales weevil, *Hylobius pales* (Herbst) (Coleoptera: Curculionidae). *Journal of Entomological Science* 29: 407-419
- Salom, S. M., Gray, J. A., Alford, A. R., Mulesk, M., Fettig, C. J. and Woods, S. A. 1996. Evaluation of natural products as antifeedants for the pales weevil (Coleoptera: Curculionidae) and as fungitoxins for *Leptographium procerum*. *Journal of Entomological Science* 31: 453-465
- Salisbury, A. N. and Leather, S. R. 1998. Migration of larvae of the large pine weevil, *Hylobius abietis* L. (Col., Curculionidae): possible predation a lesser risk than death by starvation? *Journal of Applied Entomology* 122: 295-299
- SAS Institute. 1996. *SAS/STAT user's guide, Version 6.12 4th ed.* SAS Institute, Cary, NC. USA.
- Scheibelreiter, G. K. 1976. *Investigations on the possibilities of biological control measures against Hylastes ater and Criocephalus fesus Muls. in New Zealand (Work in Europe 1973-1976).* Progress Report No. 3. Commonwealth Institute of Biological Control. European Station, Delémont, Switzerland. 4p.
- Schedl, K. E. 1968. Die Gattung *Hylastes* Er. *Anzeiger fuer Schädlingskunde* 41: 155-158
- Schmidt, R. A. and Underhill, E. M. 1974. Incidence and impact of pitch canker in slash pine plantations in Florida. *Plant Disease Reporter* 58: 451-454
- Schroeder, L. M. 1987. Attraction of the bark beetle *Tomicus piniperda* to Scots pine trees in relation to tree vigor and attack density. *Entomologia Experimentalis et Applicata* 44: 53-58
- Schroeder, L. M. 1988. Attraction of the bark beetle *Tomicus piniperda* and some other bark- and wood-living beetles to the host volatiles α -pinene and ethanol. *Entomologia Experimentalis et Applicata* 46: 203-210

- Schroeder, L. M. 1990. Duct resin flow in Scots pine in relation to the attack of the bark beetle *Tomicus piniperda* (L.) (Col., Scolytidae). *Journal of Applied Entomology* 109: 105-112
- Schroeder, L. M. and Lindelöw, Å. 1989. Attraction of scolytids and associated beetles by different and absolute amounts and proportions of α -pinene and ethanol. *Journal of Chemical Ecology* 15: 807-817
- Scott, T. M. and King, C. J. 1973. Control of *Hylobius abietis* and *Hylastes* species. Pp 108 in *Report on Forest Research for the year ended March 1973*. Forestry Commission, London.
- Scott, T. M. and King, C. J. 1974. The large pine weevil and black pine beetles. *Forestry Commission Leaflet No. 58*. Forestry Commission, London. 12p.
- Seifert, K. A. 1993. Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. Pp 141-151 in Winfield, M. J., Seifert, K. A. and Webber, J. F. (eds) *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. American Phytopathological Society, St. Paul, Minnesota.
- Selander, J. I. 1993. Survival model for *Pinus sylvestris* seedlings at risk from *Hylobius abietis*. *Scandinavian Journal of Forest Research* 8: 66-72
- Selander, J. I., Immonen, A. and Raukko, P. 1990. Resistance of naturally regenerated and nursery-raised Scots pine seedlings to the large pine weevil. *Folia Forestalia* No. 766. 44p.
- Skrzecz, I. and Moore, R. 1997. The attractiveness of pine branches infected with selected wood-colonising fungi to the large pine weevil (*Hylobius abietis*). Pp 146-152 in Gregoire, J. C., Liebhold, A. M., Stephen, F. M., Day, K. R. and Salom, S. M. (eds) *Integrating cultural tactics into the management of bark beetle and reforestation pests*. General Technical Report NE-236. US Department of Agriculture, Forest Service, Northeastern Forest Experimental Station. Radnor, Pennsylvania USDA Forest Service.
- Smalley, E. B., Raffa, K. F., Proctor, R. H. and Klepzig, K. D. 1993. Tree responses to infection by species of *Ophiostoma* and *Ceratocystis*. Pp 207-217 in Winfield, M. J., Seifert, K. A. and Webber, J. F. (eds) *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. American Phytopathological Society, St. Paul, Minnesota.

- Smith, R. H. 1964. Resin quality as a factor in the resistance of pines to bark beetles. Pp 189-196 in Gerhold, H. D., Schreiner, E. J., McDermott, R. E. and Winieski, J. A. (eds) *Breeding pest resistant trees*. Pergamon Press, Oxford.
- Smith, R. S. 1967. *Verticicladiella* root disease of pines. *Phytopathology* 57: 935-938
- Solbreck, C. 1980. Dispersal distances of migrating pine weevils, *Hylobius abietis* (Coleoptera, Curculionidae). *Entomologia Experimentalis et Applicata* 28: 123-131
- Solheim, H. 1993. Ecological aspects of fungi associated with the spruce bark beetle *Ips typographus* in Norway. Pp 235-242 in Winfield, M. J., Seifert, K. A. and Webber, J. F. (eds) *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. American Phytopathological Society, St. Paul, Minnesota.
- Southwood, T. R. E. 1962. Migration of terrestrial arthropods in relation to habitat. *Biological Review* 37: 171-214
- Stoakley, J. T. and Heritage, S. G. 1989. Application leaflet on the use of 'Gamma-Col' and 'Lindane flowable' for pre-planting treatment of young trees against *Hylobius abietis* and *Hylastes* spp. *Research Information Note* 147. Farnham, Forestry Commission Research Division. 5p.
- Stoakley, J. T. and Heritage, S. G. 1990a. Application leaflet on the use of 'Permit' and 'Permasect 25EC' for pre-planting treatment of young trees against *Hylobius abietis* and *Hylastes* spp. *Research Information Note* 177. Farnham, Forestry Commission Research Division. 5p.
- Stoakley, J. T. and Heritage, S. G. 1990b. Approved methods for insecticidal protection of young trees against *Hylobius abietis* and *Hylastes* spp. *Research Information Note* 185. Farnham, Forestry Commission Research Division. 7p.
- Stone, C. and Simpson, J. A. 1990. Species associations in *Ips grandicollis* galleries in *Pinus taeda*. *New Zealand Journal of Forestry Science* 20: 75-96
- Storer, A. J., Gordon, T. R. Wood, D. L. and Dallara, P. L. 1995. Pitch canker in California. *California Forestry Note* 110: 1-16
- Strydom, R. C., Wingfield, B. D. and Wingfield, M. J. 1997. Ribosomal DNA sequence comparison of *Leptographium lundbergii* and *L. truncatum* and neotypification of *L. lundbergii*. *Systematic and Applied Microbiology* 20: 295-300
- Swan, D. C. 1943. The bark-beetle *Hylastes ater* Payk. (Coleoptera, Scolytidae) attacking pines in South Australia. *Journal of Agriculture of South Australia* 46: 86-90
- ter Braak, C. J. F. 1987. *CANOCO- A FORTAN program for canonical community ordination by (partial) (detrended) (canonical) correspondence analysis, principle*

- component analysis (Version 2.1)*. TNO Institute of Applied Computer Science, Wageningen.
- Thompson, C. S. 1981. The climate and weather of the Taranaki region. New Zealand *Meteorological Service Miscellaneous Publication* 115(9). Wellington.
- Thompson, R. T. 1992. Observations on the morphology and classifications of weevils (Coleoptera, Curculionidae) with a key to major groups. *Journal of Natural History* 26: 835-891
- Thorin, J. and Nömmik, H. 1974. Monoterpene composition of corical resins from different clones of *Pinus sylvestris*. *Phytochemistry* 13: 1879-1881
- Thwaites, J. M. 1999. *Sapstain fungi: Colonisation and competition on radiata pine*. M.Sc. dissertation, University of Waikato, Hamilton, New Zealand. 273p.
- Tilles, D. A., Norlander G., Nordenhem, H., Eidmann, H. H., Wassgren, A. B. and Bergström, G. 1986a. Increased release of volatiles from feeding scars: A major cause of field aggregation in the pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae). *Environmental Entomology* 15: 1050-1054
- Tilles, D. A., Sjödin, K., Norlander, G. and Eidmann, H. H. 1986b. Synergism between ethanol and conifer host volatiles as attractants for the pine weevil, *Hylobius abietis* (L.) (Coleoptera: Curculionidae). *Journal of Economic Entomology* 79: 970-974
- Tomlin, E. S. and Borden, J. H. 1997a. Multicomponent index for evaluating resistance by Sitka spruce to white pine weevil (Coleoptera: Curculionidae). *Journal of Economic Entomology* 90: 704-714
- Tomlin, E. S. and Borden, J. H. 1997b. Thin bark and high density of outer resin ducts: interrelated resistance traits in Sitka spruce against white pine weevil (Coleoptera: Curculionidae). *Journal of Economic Entomology* 90: 235-239
- Tribe, G. D. 1990. Phenology of *Pinus radiata* log colonisation by the pine bark beetle *Hylastes angustatus* (Herbst) (Coleoptera: Scolytidae) in the south-western Cape Province. *Journal of the Entomological Society of Southern Africa* 53: 93-100
- Tribe, G. D. 1991a. Phenology of *Pinus radiata* log colonisation by the red-haired pine bark beetle *Hylurgus ligniperda* (Fabricius) (Coleoptera: Scolytidae) in the south-western Cape Province. *Journal of the Entomological Society of Southern Africa* 54: 1-7
- Tribe, G. D. 1991b. Phenology of three exotic pine bark beetle species (Coleoptera: Scolytidae) colonising *Pinus radiata* logs in the south-western cape province. *South African Forestry Journal* 157: 27-31

- Tunset, K., Nilssen, A. C. and Anderson, J. 1988. A new trap design for primary attraction of bark beetles and bark weevils (Col., Scolytidae and Curculionidae). *Journal of Applied Entomology* 106: 266-269
- Tunset, K., Nilssen, A. C. and Anderson, J. 1993. Primary attraction in host recognition of coniferous bark beetles and bark weevils (Col., Scolytidae and Curculionidae). *Journal of Applied Entomology* 115: 155-169
- Tuomi, J. 1992. Toward integration of plant defence theories. *Trends in Ecology and Evolution* 7: 365-367
- Vasechko, G. I. 1988. The problems that need to be solved for improvement of bark beetle control. *Journal of Applied Entomology* 106: 1-12
- Viljoen, A., Wingfield, M. J. and Marasas, W. F. O. 1994. First report of *Fusarium subglutinans* f.sp. *pini* on pine seedlings in South Africa. *Plant Disease* 78: 309-312
- Viljoen, A., Wingfield, M. J., Gordon, T. R. and Marasas, W. F. O. 1997. Genotypic diversity in a South African population of the pitch canker fungus *Fusarium subglutinans* f.sp. *pini*. *Plant Pathology* 46: 590-593
- Visser, J. H. 1986. Host odour perception in phytophagous insects. *Annual Review of Entomology* 31: 121-144
- Vité, J. P. 1961. The influence of water supply on oleoresin exudation pressure and resistance to bark beetle attack in *Pinus ponderosa*. *Contributions from the Boyce Thompson Institute* 21: 37-66
- Vité, J. P. 1967. Sex attractants in frass from bark beetles. *Science* 156: 105
- Vité, J. P. and Gara, R. I. 1962. Volatile attractants from ponderosa pine attacked by bark beetles (Coleoptera: Scolytidae). *Contributions from the Boyce Thompson Institute* 21: 251-273
- Vité, J. P. and Wood, D. L. 1961. A study on the applicability of the measurement of oleoresin exudation pressure in determining susceptibility of second growth ponderosa pine to bark beetle infestation. *Contributions from the Boyce Thompson Institute* 21: 67-78
- von Sydow, F. 1997. Abundance of pine weevils (*Hylobius abietis*) and damage to conifer seedlings in relation to silvicultural practices. *Scandinavian Journal of Forest Research* 12: 157-167
- Wainhouse, D. and Ashburner, R. 1996. The influence of genetic and environmental factors on a quantitative defensive trait in spruce. *Functional Ecology* 10: 137-143

- Watson, P. G. 1999. Influence of insecticide, wax and biofungicide treatments, applied to *Pinus sylvestris* and *Picea abies*, on the olfactory orientation of the large pine weevil, *Hylobius abietis* L. (Coleoptera: Curculionidae). *Agricultural and Forest Entomology* 1: 171-177
- Weis, A. E. and Campbell, D. R. 1992. Plant genotype: a variable factor in insect-plant interactions. Pp 75-111 in Hunter, M. D. and Price, P. W. (eds) *Effects of resource distribution on animal-plant interactions*. Academic Press. London.
- Wheeler, M. H. 1983. Comparisons of fungal melanin synthesis in ascomycetous, imperfect and basidiomycetous fungi. *Transactions of the British Mycological Society* 81: 29-36
- Whetzel, H. H. 1918. *An outline of the history of phytopathology*. W. B. Saunders Co. Philadelphia, P.A. 130p.
- White, P. R. and Hobson, K. R. 1993. Stereospecific antennal responses by the red turpentine beetle, *Dendroctonus valens* to chiral monoterpenes from ponderosa pine resin. *Journal of Chemical Ecology* 19: 2193-2202
- Wilson, W. L. and Day, K. R. 1995. The comparative effectiveness of chemical traps, and fir, spruce and larch billets, for the estimation of pine weevil (*Hylobius abietis* L.). *Journal of Applied Entomology* 119: 157-160
- Wilson, W. L., Day, K. R. and Hart, E. 1996. Predicting the extent of damage to conifer seedlings by the pine weevil (*Hylobius abietis* L.): a preliminary risk model by logistic regression. *New Forests* 12: 203-222
- Wingfield, M. J. 1983. Association of *Verticicladiella procera* and *Leptographium terrebrantis* with insects in the Lake States. *Canadian Journal of Forest Research* 13: 1238-1245
- Wingfield, M. J. and Gibbs, J. N. 1991. *Leptographium* and *Graphium* species associated with pine-infesting bark beetles in England. *Mycological Research* 95: 1257-1260
- Wingfield, M. J. and Knox-Davis, P. S. 1980. Root disease, associated with *Verticicladiella alacris*, of pines in South Africa. *Plant Disease* 64: 569-571
- Wingfield, M. J. and Marasas, W. F. O. 1980. *Ceratocystis ips* associated with *Orthomicus erosus* (Coleoptera: Scolytidae) on *Pinus* spp. in the Cape Province of South Africa. *Phytophylactica* 12: 65-69
- Wingfield, M. J. and Marasas, W. F. O. 1983. *Verticicladiella alacris*, a synonym of *V. serpens*. *Transactions of the British Mycological Society* 76: 508-510

- Wingfield, M. J. and Swart, W. J. 1994. Integrated management of forest tree diseases in South Africa. *Forest Ecology and Management* 65: 11-16
- Wingfield, M. J., Capretti, P. and Mackenzie, M. 1988. *Leptographium* spp. as root pathogens of conifers. An international perspective. Pp 113-128 in Harrington, T. C. and Cobb, F. W. Jr. (eds) *Leptographium root diseases of conifers*. American Phytopathological Society Press. St Paul, Minnesota.
- Wingfield, M. J., Swart, W. J. and von Broembsen, S. L. 1988b. Root diseases of pines and eucalypts in South Africa. Pp 563-569 in Morrison, D. J. (ed) *Proceedings of the seventh international conference on root and butt rots*. Vernon and Victoria, British Columbia, Canada, August 9-16.
- Wiseman, B.R. 1994. Plant resistance to insects in integrated pest management. *Plant Disease* 78: 927-932
- Witcosky, J. J. and Hansen, E. M. 1985. Root-colonising insects recovered from Douglas-fir in various stages of decline due to black-stain root disease. *Phytopathology* 75: 399-402
- Witcosky, J. J., Schowalter, T. D. and Hansen, E. M. 1986a. *Hylastes nigrinus* (Coleoptera; Scolytidae), *Pissodes fagiatus*, and *Steremnius carinatus* (Coleoptera: Curculionidae) as vectors of black-stain root disease of Douglas-fir. *Environmental Entomology* 15: 1090-1095
- Witcosky, J. J., Schowalter, T. D. and Hansen, E. M. 1986b. The influence of precommercial thinning on the colonization of Douglas-fir by three species of root-colonizing insects. *Canadian Journal of Forest Research* 16: 745-749
- Witcosky, J. J., Schowalter, T. D. and Hansen, E. M. 1987. Host-derived attractants for the beetle *Hylastes nigrinus* (Coleoptera: Scolytidae) and *Steremnius carinatus* (Coleoptera: Curculionidae). *Environmental Entomology* 16: 1310-1313
- Wood, D. L. 1962. Experiments on the interrelationship between oleoresin exudation pressure in *Pinus ponderosa* and attack by *Ips confusus* (Lec.) (Coleoptera: Scolytidae). *Canadian Entomologist* 94: 473-477
- Wood, D. L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behaviour of bark beetles. *Annual Review of Entomology* 27: 411-446
- Wood, S. L. 1973. On the taxonomic status of Platypodidae and Scolytidae (Coleoptera). *Great Basin Naturalist* 33: 77-90

- Wood, S. L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Naturalist Memoirs* 6: 1-1359
- Wood, S. L. 1986. A reclassification of the genera of Scolytidae (Coleoptera). *Great Basin Naturalist Memoirs* 10: 1-126
- Yazdani, R. & Nilsson, J. E. 1986. Cortical monoterpene variation in natural populations of *Pinus sylvestris* in Sweden. *Scandinavian Journal of Forest Research* 1: 85-93
- Zagatti, P., Lemperière, G. and Malosse, C. 1997. Monoterpenes emitted by the large pine weevil, *Hylobius abietis* (L.) feeding on Scots pine, *Pinus sylvestris* L. *Physiological Entomology* 22: 394-400
- Zether-Moller, O. and Rudinsky, J. A. 1967. On the biology of *Hylastes nigrinus* (Coleoptera: Scolytidae) in Western Oregon. *Canadian Entomologist* 99: 897-911
- Zimmerman, M.H. and Brown, C.L. 1977. *Trees: structure and function*. Springer-Verlag, New York. 336p.
- Zink, P. and Fengel, D. 1988. Studies on the colouring matter of blue-stain fungi. Part 1. General characterisation and the associated compounds. *Holzforschung* 42: 217-220
- Zondag, R. 1956. *Damage to young natural regeneration of P. radiata in comp. 1059 (Kaingaroa S.F.)*. New Zealand Forest Service Forest Research Institute (unpublished). Rotorua, New Zealand. 4p.
- Zondag, R. 1958. *Interim report on mortality of young natural regeneration of P. radiata in Kaingaroa S.F.* New Zealand Forest Service Forest Research Institute (unpublished). Rotorua, New Zealand. 4p.
- Zondag, R. 1964. Forest Entomology in New Zealand. *FAO/IUFRO Symposium on Internationally Dangerous Forest Diseases and Insects. Oxford, 20-30 July 1964. Meeting No. II-III*. 8p.
- Zondag, R. 1965. Insect pests of forest nurseries and young plantations in New Zealand. *Proceedings of the Twelfth International Congress of Entomology, London, 1964*: 675
- Zondag, R. 1968. Entomological problems in New Zealand Forests. *Proceedings of the New Zealand Ecological Society* 15: 10-14
- Zondag, R. 1976a. *Phloeosinus cupressi* Hopkins (Coleoptera: Scolytidae) Cypress Bark beetle. *Forest and Timber Insects in New Zealand No. 3*. Forest Research Institute, New Zealand Forest Service. 4p.

- Zondag, R. 1976b. *Pteromalid parasites for the control of Hylastes ater. Part 1 breeding and releases until May 1976*. New Zealand Forest Service, Forest Research Institute, Forest Entomology Report No. 44 (unpublished). Rotorua, New Zealand. 7p.
- Zondag, R. 1979. Breeding of the clerid *Thanasimus formicarius* for the control of the bark beetles *Hylastes ater* and *Hylurgus ligniperda* in New Zealand. *New Zealand Journal of Forestry Science* 9: 125-132
- Zondag, R. 1982. Insects of the exotic forest of the central North Island. *New Zealand Entomologist* 7: 276-280
- Zondag, R., Bain, J. and Faulds, W. 1976. *Pteromalid parasites for the control of Hylastes ater. Part 2. The breeding from May to end of October 1976*. New Zealand Forest Service, Forest Research Institute, Forest Entomology Report No. 44 (unpublished). Rotorua, New Zealand. 3p.
- Zumr, V and Starý, P. 1994. Monitoring of seasonal occurrence of *Hylobius abietis* (L.) (Col., Curculionidae) in different forest environments of a model area. *Journal of Applied Entomology* 118: 361-364
- Zumr, V and Starý, P. 1995. LATEX paint as an antifeedant against *Hylobius abietis* (L.) (Col., Curculionidae) on conifer seedlings. *Anzeiger für Schadlingskunde, Pflanzenschutz, Umweltschutz* 58: 42-43

APPENDIX 1.

Site	Forest	Compartment/OP ID
1	Kaingaroa	1047
2	Kaingaroa	1312
3	Kaingaroa	189
4	Kinleith	27328
5	Kinleith	28987
6	Kinleith	29034
7	Kinleith	29075
8	Kinleith	29083
9	Kinleith	32027
10	Kaingaroa	503
11	Kaingaroa	437
12	Kaingaroa	91
13	Kaingaroa	80
14	Kaingaroa	410
15	Kaingaroa	854
16	Kaingaroa	1005
17	Kinleith	29825
18	Kinleith	30859
19	Kinleith	30958
20	Kaingaroa	1016
21	Kaingaroa	171
22	Kinleith	27564
23	Kinleith	29822
24	Kinleith	Rawhiti Rd
25	Kinleith	80404
26	Kaingaroa	1114
27	Kinleith	29822
28	Kinleith	80404
29	Kaingaroa	908
30	Kaingaroa	1044
31	Kaingaroa	11
32	Kinleith	30887
33	Kinleith	30877
34	Kinleith	35427
35	Kinleith	29007